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DEPARTMENT OF AGRICULTURE.

SEVENTEENTH REPORT

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Ninth and Tenth Reports of the Director of Veterinary Education and Research, April, 1923.

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P. J. du TOIT,
Director of Veterinary Services.

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Protozoal Diseases.

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B. S. Parkin ... Styrylquinoline No. 314 in Trypanosomiases.

B. S. Parkin ... A Note on the Diagnosis of Bovine Trypanosomiases.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Immunity in East Coast Fever.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services and Animal Industry.

In a paper read before the South African Association for the Advancement of Science in July, 1928, a brief account was given of some experiments that were undertaken with the object of determining whether it was possible to break down the immunity of animals which had recovered from East Coast fever. It was explained that the immunity of such animals *could* be broken down, in a small percentage of cases, if they were exposed to a natural infection, that is, if they were bitten by infected ticks. That fact was well known. But the point to be decided by the experiments was whether *in the absence of infected ticks*, a breakdown in immunity could be brought about.

The importance of getting a definite reply to that question was also explained in the paper referred to. If a breakdown in immunity after recovery from East Coast fever were possible, in the same way that a relapse may occur in malarial patients *in the absence of infected mosquitoes*, or in animals that have recovered from piroplasmosis or anaplasmosis *in the absence of infected ticks*, then such a recovered animal would constitute a grave source of danger. The position would then be that such an animal might get a relapse in a clean area and infect some ticks which could then start a new outbreak of the disease. That such an explanation is often advanced by farmers and others to account for a new outbreak of East Coast fever is well known. And, indeed, this explanation not infrequently appears to be the most plausible in cases where the disease reappears in an area which had been considered clean for a number of years. But, so far, no facts have been advanced in support of this theory. On the contrary, all the experimental evidence which we possess, is in support of the view that the animal which recovers from East Coast fever cannot (in the absence of infected ticks) get a relapse, and hence is not a source of danger for a fresh infection. A large volume of field experience also lends support to this view; one example may be quoted here. The Pretoria district was very badly infected with East Coast fever during the years 1919 and 1920. The disease was finally stamped out in 1923 and, although there must have been a large number of recovered animals in the district at the time, there has been no recrudescence of the disease since then. The same applies to other districts.

In the experiments referred to above, it was attempted in a variety of ways to break down the immunity of animals which had recovered from East Coast fever. "Provocatives" were injected into

some animals in the hope of "stirring up" any "latent infection" which might be present; other animals were subjected to severe bleedings with the same object in view; others, again, were subjected to various operations (splenectomy or fractures); and yet others were infected with various diseases (contagious abortion, trypanosomiasis, heartwater). In all these cases the results were entirely negative as far as East Coast fever was concerned, although other parasites (*Piroplasma bigeminum*, *Anaplasma marginale*, *Theileria mutans*) appeared in the blood of the animals which were treated in these ways.

The conclusion drawn from these experiments was that it did not appear possible to produce a relapse in an animal recovered from East Coast fever, and that new outbreaks of the disease must invariably be ascribed to infected ticks.

Additional evidence in support of this conclusion will be supplied in the following pages, but before doing so it is considered necessary to give a short account of these experiments, partly for the sake of completeness and ease of reference, and partly because in the publication already referred to experimental data were not included.

Experiment 1 (S. 3102 and 3134).

Injection of Provocatives.

Object (in this and the following experiments): To produce a relapse in animals which had recovered from East Coast fever.

Method: Intravenous injection of substances known to be capable of producing a relapse in other protozoal diseases.

Sterile milk and *egg albumen*, which have been used as provocatives in malaria, were used in one case, and *trypanblue*, which was known to bring about a relapse of *Anaplasma marginale* and *Theileria mutans*, in two other cases.

(1) *Ox* 1627, which had recovered from East Coast fever in March, 1926, received on—

16.6.27 50 c.c. sterile milk intravenously.
21.6.27 100 c.c. ,, ,, ,,
24.6.27 80 c.c. diluted egg albumen intravenously.
30.6.27 80 c.c. ,, ,, ,, ,,
4.7.27 100 c.c. ,, ,, ,, ,,

Result: Negative, apart from a slight shock after the second injection. East Coast fever parasites were never seen.

(2) *Bull* 894, which had recovered from East Coast fever in December, 1924, received on—

15.7.27 100 c.c. 1% trypanblue intravenously.
17.7.27 100 c.c. ,, ,, ,,
19.7.27 150 c.c. ,, ,, ,,
21.7.27 150 c.c. ,, ,, ,,
23.7.27 200 c.c. ,, ,, ,,
25.7.27 200 c.c. ,, ,, ,,
27.7.27 250 c.c. ,, ,, ,,
28.7.27 250 c.c. ,, ,, ,,
29.7.27 250 c.c. ,, ,, ,,
30.7.27 250 c.c. ,, ,, ,,

1.8.27– 6.8.27 daily 300 c.c. 1% trypanblue intravenously.
8.8.27– 12.8.27 ,, 300 c.c. ,, ,, ,,
15.8.27– 20.8.27 ,, 300 c.c. ,, ,, ,,
22.8.27– 27-8.27 ,, 300 c.c. ,, ,, ,,
29.8.27– 30.8.27 ,, 300 c.c. ,, ,, ,,
1.9.27– 3.9.27 ,, 300 c.c. ,, ,, ,,
6.9.27– 10.9.27 ,, 300 c.c. ,, ,, ,,
12.9.27– 17.9.27 ,, 300 c.c. ,, ,, ,,
19.9.27– 24.9.27 ,, 300 c.c. ,, ,, ,,
26.9.27– 28.9.27 ,, 300 c.c. ,, ,, ,,
10.10.27–14.10.27 ,, 300 c.c. ,, ,, ,,
14.11.27 300 c.c. 1% trypanblue intravenously.
16.11.27 300 c.c. ,, ,, ,,
Altogether 18,400 c.c. 1% solution or 184 grams trypanblue.

Result : Soon after the beginning of the injections, *Theileria mutans* and *Anaplasma marginale* appeared in the blood, but East Coast fever parasites were never seen.

The animal showed signs of distress after the first few injections, but these symptoms did not appear later on. After the 28th September, 1927, the injections were discontinued for a time on account of general weakness of the animal. Later on it recovered completely. It may be added that when this animal died about 9 months later (12.8.28), its tissues still showed an intense blue colouration as a result of the trypanblue injections.

(3) *Ox* 1627 (see above), which had recovered from East Coast fever in March, 1926, received on—

11.7.27 100 c.c. 1% trypanblue intravenously.
13.7.27 100 c.c. ,, ,, ,,
15.7.27 100 c.c. ,, ,, ,,
17.7.27 100 c.c. ,, ,, ,,
19.7.27 150 c.c. ,, ,, ,,
21.7.27 150 c.c. ,, ,, ,,
23.7.27 200 c.c. ,, ,, ,,
25.7.27 200 c.c. ,, ,, ,,
27.7.27 to 30.7.27 daily 250 c.c. trypanblue intravenously.
1.8.27 to 6.8.27 ,, 300 c.c. ,, ,,
8.8.27 to 12.8.27 ,, 300 c.c. ,, ,,
15.8.27 to 20.8.27 ,, 300 c.c. ,, ,,
22.8.27 to 27.8.27 ,, 300 c.c. ,, ,,
29.8.27 to 1.9.27 ,, 300 c.c. ,, ,,
6.9.27 to 10.9.27 ,, 300 c.c. ,, ,,
12.9.27 to 17.9.27 ,, 300 c.c. ,, ,,
19.9.27 to 20.9.27 ,, 300 c.c. ,, ,,
Altogether 14,100 c.c. 1% solution, or 141 grams trypanblue.

Result : This ox died three days after the last injection.

Theileria mutans and *Anaplasma marginale* appeared in its blood, but no East Coast fever parasites were seen.

Experiment 2 (S. 3102 and 3200).

Production of Anaemia.

Method : Two methods of producing anaemia were tried, firstly by withdrawing large quantities of blood from the animals and secondly by injecting *Ichthargan*, a substance known to produce anaemia.

(1) *Heifer* 870, which had recovered from East Coast fever in December, 1924, was bled as follows:—

15.6.27	5	litres	blood withdrawn.
20.6.27	5	,,	,, ,,
24.6.27	5	,,	,, ,,
27.6.27	5	,,	,, ,,
30.6.27	5	,,	,, ,,
4.7.27	5	,,	,, ,,
7.7.27	5	,,	,, ,,
11.7.27	9	,,	,, ,,
14.7.27	5	,,	,, ,,
21.7.28	7	,,	,, ,,
25.7.27	5	,,	,, ,,
29.7.27	5	,,	,, ,,
2.8.27	5	,,	,, ,,
15.8.27	5	,,	,, ,,
19.8.27	5	,,	,, ,,
22.8.27	5	,,	,, ,,
29.8.27	5	,,	,, ,,
2.9.27	5	,,	,, ,,
9.9.27	5	,,	,, ,,
12.9.27	5	,,	,, ,,
16.9.27	5	,,	,, ,,
19.9.27	5	,,	,, ,,
23.9.27	5	,,	,, ,,
26.9.27	5	,,	,, ,,
30.9.27	5	,,	,, ,,
10.10.27	5	,,	,, ,,
21.10.27	5	,,	,, ,,
24.10.27	5	,,	,, ,,
28.10.27	5	,,	,, ,,

Altogether 151 litres in the course of about 4 months.

Result : The animal showed severe anaemic changes in the blood, and other blood parasites appeared (*Theileria mutans* and *Anaplasma marginale*), but there was never a suspicion of East Coast fever.

(2) *Ox* 1109, which recovered from East Coast fever in December, 1926, was treated as follows:—

15.6.27	5	litres	blood withdrawn.
20.6.27	5	,,	,, ,,
24.6.27	5	,,	,, ,,
27.6.27	5	,,	,, ,,
30.6.27	5	,,	,, ,,
4.7.27	5	,,	,, ,,
7.7.27	5	,,	,, ,,
11.7.27	5	,,	,, ,,
14.7.27	5	,,	,, ,,
21.7.27	7	,,	,, ,,
25.7.27	5	,,	,, ,,
29.7.27	5	,,	,, ,,
2.8.27	5	,,	,, ,,
15.8.27	5	,,	,, ,,
19.8.27	5	,,	,, ,,
22.8.27	5	,,	,, ,,
29.8.27	5	,,	,, ,,
2.9.27	5	,,	,, ,,
9.9.27	5	,,	,, ,,
12.9.27	5	,,	,, ,,
16.9.27	5	,,	,, ,,
19.9.27	5	,,	,, ,,
23.9.27	5	,,	,, ,,
26.9.27	5	,,	,, ,,
30.9.27	5	,,	,, ,,

Altogether 127 litres were withdrawn in the course of 3½ months.

Result : This animal died one day after the last bleeding. Its blood had shown severe anaemic changes, and *Theileria mutans* and *Anaplasma marginale* were present, but there was no sign of East Coast fever.

(3) *Heifer* 1199, which had recovered from East Coast fever in December, 1924, received the following injections:—

7.9.27	100 c.c.	1%	solution	Ichthargan	intrav.
9.9.27	100 c.c.	,,	,,	,,	,,
12.9.27	100 c.c.	,,	,,	,,	,,
14.9.27	100 c.c	,,	,,	,,	,,
16.9.27	100 c.c.	,,	,,	,,	,,
19.9.27	100 c.c.	,,	,,	,,	,,
21.9.27	100 c.c.	,,	,,	,,	,,
23.9.27	100 c.c.	,,	,,	,,	,,
26.9.27	100 c.c.	,,	,,	,,	,,
28.9.27	100 c.c.	,,	,,	,,	,,
30.9.27	100 c.c.	,,	,,	,,	,,
10.10.27	100 c.c.	,,	,,	,,	,,
12.10.27	100 c.c.	,,	,,	,,	,,
14.10.27	100 c.c.	,,	,,	,,	,,
17.10.27	100 c.c.	,,	,,	,,	,,

Altogether 15 grams of Ichthargan.

Result : The blood of the animal became very anaemic, but there was no sign of East Coast fever. Swellings developed at the seat of injection, and the injections were discontinued because of these.

(4) *Ox* 2054, which had recovered from East Coast fever in March, 1927, received the following injections:—

7.9.27	75 c.c.	1%	solution	Ichthargan	intrav.
9.9.27	75 c.c.	,,	,,	,,	,,
12.9.27	75 c.c.	,,	,,	,,	,,
14.9.27	75 c.c.	,,	,,	,,	,,
16.9.27	75 c.c.	,,	,,	,,	,,
19.9.27	75 c.c.	,,	,,	,,	,,
21.9.27	75 c.c.	,,	,,	,,	,,
23.9.27	75 c.c.	,,	,,	,,	,,
26.9.27	100 c.c.	,,	,,	,,	,,
28.9.27	100 c.c.	,,	,,	,,	,,
30.9.27	100 c.c.	,,	,,	,,	,,
10.10.27	100 c.c.	,,	,,	,,	,,
12.10.27	100 c.c.	,,	,,	,,	,,
14.10.27	100 c.c.	,,	,,	,,	,,

Altogether 12 grams of Ichthargan. The injections were discontinued on account of the large swelling on the neck of the animal. During the course of the injections the blood became very anaemic, but no parasites of East Coast fever were ever seen.

Experiment 3 (S. 2061).

Splenectomy.

Methods: In animals which have recovered from East Coast fever the spleen was removed in order to ascertain whether the parasites would reappear after the operation in the same way that many other parasites (*Piroplasma bigeminum, Theileria mutans, Anaplasma marginale, Piroplasma caballi, Nuttallia equi, Piroplasma ovis, Theileria recondita, Anaplasma ovis*) reappear after splenectomy in animals which had previously passed through these infections.

(1) *Heifer* 870, which had recovered from East Coast fever in January, 1925, was splenectomized on the 30th April, 1925.

(2) *Bull* 894, which also had recovered from East Coast fever in January, 1925, was splenectomized on the 17th March, 1925.

Result : In neither animal did the parasites of East Coast fever reappear after the operation. The animals were kept under close observation for more than three years (until they both died in another experiment), but there was never a suspicion of a relapse of East Coast fever.

Experiment 4 (S. 3130).

Bodily Injury.

Method : The animals were brought under complete narcosis, and a bone in one of the limbs was then fractured to see whether such a serious injury would result in a relapse of East Coast fever. The reason why this experiment was undertaken was that, under field conditions, East Coast fever had more than once been diagnosed in an animal that was reported to have broken a limb and to have lain on the veld for several days in a helpless condition.

(1) *Ox* 2054, which had recovered from East Coast fever in March, 1927, was operated on on the 15th July, 1927, when the right metatarsus was broken. The limb was set in plaster of paris, and the animal made a complete and rapid recovery.

(2) *Heifer* 1199, which had recovered from East Coast fever in February, 1927, was treated in a similar way on the 4th August, 1927. An ordinary bandage with wooden splints was applied to the broken limb, and this bandage was removed after 4 days. This animal also made a rapid recovery.

(c) *Ox* 825 had recovered from East Coast fever in July, 1925. On the 22nd August, 1927, its right metatarsus was fractured under narcosis and the leg was left unbandaged. There was a slight rise of temperature for a few days during the second week after the operation, but otherwise the animal made an uneventful recovery.

Result : In none of these three animals did East Coast fever parasites appear at any stage after the operation.

Experiment 5 (S. 3110).

Attempt to Produce Peritonitis.

Method : Bacterial cultures and an irritating material (turpentine) were injected into the peritoneal cavity of an animal which had recovered from East Coast fever. The object was to produce peritonitis and to ascertain whether this would bring about a relapse to East Coast fever.

Bull 1894, which had recovered from East Coast fever in March, 1927, received the following treatment:—

22.6.27 intraperitoneal injection of 20 c.c. of a 24-hour-old broth culture of *Bacillus nephritidis equi.*

18.7.27 intraperitoneal injection of 60 c.c. of an emulsion containing *B. coli, Bac. paratyphosum* A. and B., and *Bac. enteritidis.*

3.8.27 intraperitoneal injection of 1 c.c. turpentine.

15.8.27 3 c.c. turpentine.

22.8.27 10 c.c. turpentine.

17.9.27 25 c.c. of an emulsion containing *Staph. aureus, St. albus, St. citreus, Bac. pyocyaneus, Sarcina lutea,* and *Corynebact. ovis.*

Result : After the second injection the animal's temperature rose to 106° F. and remained high for a few days. After that the temperature was somewhat irregular, but there were never definite signs of a peritonitis.

In the blood a few small piroplasms (*Theileria mutans*) were seen from time to time, but there was never a suspicion of East Coast fever.

EXPERIMENT 6 (S. 3131).

Attempt to Produce Abortion and Metritis.

Method : An attempt was made to procure abortion in a pregnant cow which had recovered from East Coast fever, first by manual interference, and then by infection with contagious abortion bacteria.

When abortion did take place the afterbirth was not removed so that metritis should develop. To encourage this, a mixture of various bacterial cultures was injected into the uterus. There was a discharge for about a fortnight, after which the cow recovered completely.

The reason for this experiment was that, under field conditions, cases had been recorded in which East Coast fever had been diagnosed in cows which had aborted and then contracted chronic metritis. It was thought that such a condition might possibly bring about a relapse of East Coast fever.

The history of the experimental case was briefly as follows:—

Cow 725 recovered from East Coast fever in May, 1925; pregnant since October, 1926.

15.7.27 portion of cervical plug removed, cervical canal dilated, saline solution injected into uterus.

8.8.27 subcutaneous injection of contagious abortion culture (five-days' growth).

8.8.27 to 13.8.27 high temperature.

5.9.27 aborted.

14.9.27 afterbirth expelled.

17.9.27 injected into uterus 25 c.c. of mixed culture of *Staph. aureus, St. albus, St. citreus, B. pyocyaneus, Sarcina lutea,* and *Corynebact. ovis.*

24.9.27 to 1.10.27 discharge from vagina.

Result : Blood and gland smears of the animal were examined regularly and over a long period, but there was never a sign of East Coast fever.

EXPERIMENT 7 (S. 3127).

Infection with Trypanosomes.

Method : Animals which had recovered from East Coast fever were infected with Trypanosomes to see if this infection would break down the immunity to East Coast fever and cause the parasites to reappear.

(1) *Ox* 715, which had recovered from East Coast fever in October, 1925, was infected with *Trypanosoma congolense* from a guinea-pig op the 9th July, 1927. Trypanosomes appeared in the blood of the ox a few days later and remained present until death took place on the 10th February, 1928.

(2) *Heifer* 893, which was splenectomized on the 15th December, 1924, and survived an East Coast fever infection in May, 1925, was infected with *Trypanosoma congolense* on the 9th July, 1927. The trypanosomes became very numerous in the blood and the animal lost rapidly in condition. It died on the 6th October, 1927 (i.e. after three months).

(3) *Heifer* 1199, which had recovered from East Coast fever in February, 1927, was exposed to the bites of tsetse-flies (*Glossina pallidipes*) from Zululand in January, 1928. *Trypanosoma vivax* appeared in its blood a few weeks later (see Du Toit, 1929). The animal was kept under observation until it died on the 12th August, 1928.

Result : A fatal infection of trypanosomiasis (*Tryp. congolense* in two cases; *Tryp. vivax* in one case) failed to break down the immunity to East Coast fever in three animals. After the death of these animals, their various organs were examined for developmental stages of *Theileria parva* (Koch's bodies), but none were found.

EXPERIMENT 8 (S. 3098).

Infection with Heartwater.

Method : Animals which had recovered from East Coast fever were infected with heartwater to see if this infection would cause a relapse of East Coast fever.

(1) *Ox* 715, which had recovered from East Coast fever in October, 1925, was infected with heartwater from a sheep on the 11th June, 1927. A mild attack of heartwater began about 18 days after the injection. No parasites were seen in the blood.

(2) *Ox* 1012, which had recovered from East Coast fever in April, 1926, was infected with heartwater from a sheep on the 11th June, 1927. A severe reaction began 16 days after the injection, and the animal died on the 21st day (2nd July, 1927).

Result : No parasites were seen during the reaction, and after death no parasites could be found in the organs of 1012.

In Experiment 9 (see below) it will be noticed that quite a number of East Coast fever immune animals died of heartwater. In none of these animals were East Coast fever parasites found during these reactions or after death, nor was there any suspicion that any of them ever had a relapse of East Coast fever.

The above experiments seem to prove fairly conclusively that it is not possible to produce a relapse in an animal which has recovered from East Coast fever. In no single case did the parasites of East Coast fever reappear after recovery, whatever hardships the recovered animals were exposed to. *It would seem, therefore, that recovered animals can be taken, with complete safety, into a clean area and that such animals could never cause a fresh outbreak of the disease.*

As stated at the beginning of this article, the bulk of the evidence which has been collected over the last 25 years in the field, supports this view. Where every other source of infection (e.g. infected ticks) can be ruled out, no fresh infection need be feared, however many recovered animals may be present. In cases where a fresh outbreak of the disease is ascribed to a recovered animal by the farmers it is usually possible to trace the infection to an extraneous source or, at any rate, such a source cannot be excluded. The experimental results recorded above seem to have brought further very strong evidence in vindication of the innocence of the recovered animal.

There seems to be only one argument which can legitimately be urged against this conclusion. It may be said that, even though in the above experiments a relapse was never *observed*, and the parasites of East Coast fever were never *seen* in the blood or glands, yet the parasites may have been there in rare numbers, so rare that they escaped detection by the ordinary examination under the microscope. In this case—it may be argued—ticks, had they been present on the animal, might have been able to pick up the infection and transmit it to other animals.

Such an objection would be perfectly valid. In "redwater" (*Piroplasma bigeminum* infection) or anaplasmosis, it is well known that ticks *can* pick up the infection, even though the animals do not show the infection in the blood.

In order to meet this objection, an experiment was devised to determine whether ticks could infect themselves on East Coast fever recovered animals, and then transmit the disease to other animals. As will be seen from the following description, it was endeavoured to make the experiment as natural, and the conditions as severe, as possible.

EXPERIMENT 9. (S. 3460.)

Tick Transmission.

Object of the Experiment.—To detemine whether East Coast fever is liable to appear in a herd of cattle consisting of recovered and susceptible animals running on a farm which is heavily infested with the natural transmitter of the disease (*Rhipicephalus appendiculatus*).

Method.—A site was selected on a farm "Kaalplaas," about five miles from Onderstepoort, and here an area of about 100 morgen (200 acres) was fenced in. To ensure effective isolation of the animals a double fence was erected right round the camp, the two parallel fences running about 10 yards apart (see Figure 3). This camp was situated more or less in the centre of the farm, the boundary of which was also very securely fenced, so that the camp was actually surrounded by three concentric fences.

The type of vegetation in this camp was such as one might find on any typical East Coast fever farm (see Figures 1 and 2). There was sufficient grass for the animals and also sufficient trees to afford shelter.

In this camp *tick life* was very abundant (see later). The brown tick (*Rhipicephalus appendiculatus*) and the red tick (*Rhipicephalus evertsi*), the two common carriers of East Coast fever, were particularly frequent, but also the blue tick (*Boophilus decoloratus*), the bont tick (*Amblyomma hebraeum*) and the bont-leg tick (*Hyalomma aegyptium*) were by no means rare.

Into this camp nine animals which had recovered from East Coast fever and eight susceptible animals were brought on the 2nd April, 1928. At the time of writing (middle of March, 1931) this experiment has, therefore, been running for almost exactly three years. Further animals, both immune and suspectible, were added during the following months (for details see below). Altogether 25 immune and 23 suspectible animals were introduced into the camp and a further 10 calves (suspectible) were born in the camp in the course of the experiment. Of these 58 animals no less than 28 died (or were killed *in extremis*) during the experiment, one was returned to Onderstepoort, and 29 are still alive to-day.

The number of animals may appear to be rather small, but the grazing was not too good and the camp could not carry more than about 30 animals at a time all the year round. As a matter of fact during the winter of 1930 the camp had to be extended in order to provide sufficient grazing.

The ideas underlying this experiment will be obvious from a consideration of the remarks made above in connection with experiments 1 to 8.

Animals which had recovered from East Coast fever were exposed here to all the rigours of the climate and to various infections. They were, furthermore, exposed to the bites of innumerable ticks, chief amongst which were the transmitters of East Coast fever. If at any time during the three years, any one of these animals had shown a relapse, however slight, one could reasonably suppose that the ticks would have "picked up" the infection. They would then, of necessity, have transmitted the infection to the suspectible animals and the disease would have been diagnosed.

In order to maintain a strict control over these animals an assistant was sent to the camp every day. *Temperatures* were then taken of all the animals, and whenever a temperature was above 103° F. *blood and gland smears* were made and brought to the Laboratory for examination. Once a week a more senior assistant was sent out to check the temperatures and to make blood and gland smears of all the animals.

Whenever an animal was reported to be seriously ill or to have died, a professional officer went out to make a careful examination or perform an autopsy. In the latter case smears were made of all the organs, so that a careful search for East Coast fever parasites could be made. During the three years at least 10,000 smears must have been made and examined in connection with this experiment.

Details of the Experiment.

In the following table a few details are given of the *immune* animals which were used in this experiment:—

Table 1.

No. of Animal.	Sex.	Date of Introduction into Camp.	Recovery from E.C. F. (1).	Source of Infection.	Result of Exposure at Kaalplaas.		
					Infections which Appeared.	Fate of Animal.	Cause of Death.
725	Heifer.	2.4.28	May, 1925.....	Infected ticks batch 687.....	*A. marg., Th. mut.* (2).....	Died 21.8.30...	Dystokia.
825	Ox....	„	July, 1925.....	Infected ticks batch 694.....	H.W., *P. big., Th. mut*.....	Still alive.....	—
870	Heifer.	„	January, 1925.	Intravenous injection of gland and spleen pulp (3)	*P. big., A. marg*.....	Died 3.5.28.....	Piroplasmosis.
894	Bull...	„	„	„ „ „	*P. big.*, H.W.....	Died 21.5.28....	Piroplasmosis and Heartwater.
1199	Heifer.	„	February, 1927	Subcutaneous and intradermal injection of blood (3)	*T. vivax* (4).....	Died 12.8.28....	Trypanosomiasis.
1894	Bull...	„	March, 1927...	„ „ „	*A. marg., Th. mut*.....	Killed *in extremis*, 18.10.30	General debility.
2054	Ox....	„	„ ...	„ „ „	*P.big., A.marg., Th.mut.*, probably H.W.	Still alive.....	—
2076	Ox....	„	„ ...	„ „ „	*A. marg., Th. mut.*, probably H.W....	„ „	—
2098	Bull...	„	„ ...	Subcutaneous injection of blood.....	*P. big., Th. mut.*, H.W.....	Died 9.5.28.....	Piroplasmosis and Heartwater.
2193	Ox....	1.8.28	May, 1927.....	Intraperitoneal injection of blood....	*A. marg., P. big., Th. mut.*, prob. H.W.	Still alive.....	—
629	Cow...	2.8.28	November, 1927	Exposure on E.C.F. farm Kindergoed (5)	*A. marg., Th. mut*.....	Killed *in extremis*, 20.7.29	Debility.
631	Cow...	„	„	„ „ „	*Th. mut*.....	Died 28.10.29...	Cachexia.
1863	Cow...	„	January, 1928.	„ „ „	*P. big., Th. mut*.....	Still alive.....	—
2170	Heifer.	„	November, 1927	„ „ „	*P. big., A. marg., Th. mut*.....	„ „	—
2184	Bull...	„	January, 1928.	„ „ „	*P. big., A. marg., Th. mut*.....	„ „	—
2202	Heifer.	„	„	„ „ „	*Th. mut*.....	„ „	—
2206	Bull...	„	November, 1927	„ „ „	*P. big., A. marg., Th. mut*.....	„ „	—
2502	Bull...	„	? (6)	Exposure on E.C.F. farm Kindergoed	*Th. mut*.....	„ „	—
2503	Cow...	„	?	„ „ „	*A. marg., Th. mut*.....	„ „	—
2505	Ox....	„	?	„ „ „	*A. marg., Th. mut*.....	„ „	—
2511	Ox....	„	?	„ „ „	*Th. mut*.....	„ „	—
2512	Ox....	„	?	„ „ „	*P. big., Th. mut*.....	„ „	—
2513	Cow...	„	?	„ „ „	*P. big., An. marg., Th. mut*.....	Killed *in extremis*, 8.6.29	Cachexia.
2521	Ox.....	„	October, 1927..	„ „ „	*An. marg., Th. mut*.....	Still alive.....	—
2783	Heifer.	24.12.28	August, 1928..	Infected ticks batch 953.....	*P. big., A. marg., Th. mut*.....	Killed *in extremis*, 3.5.29.	Cachexia.

(1) In the majority of the animals mentioned in this table the immunity, after recovery, was tested by placing infected ticks on the animals. In some cases this was repeated up to 8 or 9 times. Only in two cases (Nos. 870 and 894) did the animals show a second, mild E.C.F. (East Coast Fever) reaction after such a test. It is interesting to note that both these animals had been splenectomized.

(2) The abbreviations used in this column are: *A. marg.* for *Anaplasma marginale*, *Th. mut.* for *Theileria mutans*, H.W. for Heartwater, *P. big.* for *Piroplasma bigeminum*, *T. vivax* for *Trypanosoma vivax*.

(3) These experiments were described by Theiler and Du Toit (1929).

(4) Heifer 1199 had been bitten by infected tsetse flies (*Glossina pallidipes*) from Zululand on the 30.1.1928 and subsequent days.

(5) A description of this extremely heavily infected farm and the experiments conducted on it, will be found in the article by Du Toit and Viljoen (1929).

(6) Animals 2502, 2503, 2505, 2511, 2512, and 2513 were brought from Kindergoed to Onderstepoort in May, 1928. They were purchased from the natives at Kindergoed and were stated to be immune against East Coast fever. These cattle had run in infected camps where the mortality from East Coast fever amongst susceptible animals was nearly 100 per cent. There was, therefore, no reason to doubt their immunity.

Table 2 gives similar details for the *susceptible animals* introduced into the experiment:—

Table 2.

No. of Animal.	Sex.	Date of Introduction into Camp.	Result of Exposure at Kaalplaas.		Cause of Death.
			Infections which Appeared.	Fate of Animal.	
1489	Heifer	2.4.28	*Th. mut*	Died 7.5.28	Heartwater.
1609	Heifer	,,	*P. big.*, *Th. mut.*, H.W.	Still alive	—
1846	Bull	,,	*Th. mut*	Died 13.6.28	Heartwater.
1852	Heifer	,,	*P. big.*, *A. marg.*, *Th. mut.*, prob. H.W.	Still alive	—
1856	Bull	,,	*P. big.*, *Th. mut*	Died 15.5.28	Heartwater.
2039	Heifer	,,	*A. marg.*, *Th. mut.*, prob. H.W.	Still alive	—
2466	Heifer	,,	*P. big.*, *A. marg.*, *Th. mut*	,, ,,	—
2477	Heifer	,,	*Th. mut*	Died 2.5.28	Heartwater.
1034	Ox	9.5.28	—	Returned to Onderstepoort, 23.5.28	—
2207	Ox	1.8.28	*P. big.*, *A. marg.*, *Th. mut*	Killed *in extremis*, 8.6.29	Cachexia.
1878	Ox	2.11.28	*P. big.*, *A. marg.*, *Th. mut*, *Spir. theileri*, H.W.	Still alive	—
2322	Ox	,,	*P. big.*, *A. marg.*, *Th. mut*	Died 27.12.28	Heartwater.
2336	Ox	,,	*P. big.*, *A. marg.*, *Th. mut*	Killed *in extremis*, 24.1.29	Heartwater.
2418	Ox	,,	*P. big.*, *A. marg.*, *Th. mut*	Still alive	—
2491	Ox	24.12.28	*P. big.*, *A. marg.*, *Th. mut.*, and H.W.	Died 4.8.29	Cachexia.
3515	Heifer	18.11.29	*P. big.*, *A. marg.*, *Th. mut*	Died 29.12.29	Piroplasmosis.
3528	Heifer	,,	*P. big.*, *A. marg.*, *Th. mut*	Died 19.11.30	Cachexia.
416	Ox	12.3.30	*T. congolense*	Died 15.5.30	Trypanosomiasis.
2784	Ox	,,	—	Still alive	—
2789	Heifer	,,	*A. marg*	,, ,,	—
2800	Ox	,,	*Th. mut*	Died 27.10.30	Uncertain.
3508	Heifer	,,	*A. marg.*, *T. cong*	Died 13.4.30	Trypanosomiasis.
3520	Heifer	,,	*P. big.*, *Th. mut.*, *T. congolense*	Died 21.5.30	Trypanosomiasis.

Table 3 gives the necessary details of the *calves* which were born in the camp during the experiment:—

Table 3.

Calf No.	Sex.	Date of Birth.	No. of Mother.	Infections which Appeared.	Fate of Animal.	Cause of Death.
2881	Heifer.....	13. 1.29	2503	*P. big.*, *A. marg.*, *Th. mut.*, and H.W.....	Still alive.....	—
2884	Bull.....	18. 1.29	2513	*P. big.*, *A. marg.*, *Th. mut*.....	Died 15.6.29.....	Inanition.
2901	Bull.....	27. 2.29	629	*P. big.*, *A. marg.*, *Th. mut*.....	Died 11.6.29.....	,,
3550	Heifer.....	6. 6.29	631	*P. big.*, *Th. mut*.....	Still alive.....	—
3687	Bull.....	7.12.29	2521	*P. big.*, *A. marg*.....	,, ,, ...	—
4222	Bull.....	1. 9.30	1852	*P. big*.....	,, ,, ...	—
4277	Heifer.....	24.11.30	2202	*P. big.*, *A. marg*.....	,, ,, ...	—
4282	Bull.....	29.11.30	2503	*P. big.*, *Th. mut*.....	Died 30.1.31.....	Piroplasmosis.
4294	Bull.....	18.12.30	2170	*P. big*.....	Died 19.1.31.....	,,
4310	Heifer.....	22. 1.31	1863	*P. big*.....	Still alive.....	—

Discussion of Results.

The experiment was undertaken with the object of finding out whether East Coast fever would appear when immune and susceptible animals were run together in a camp heavily infested with ticks. The results, as far as this object is concerned, was *entirely negative*. There was never a suspicion or East Coast fever, either in an immune or in a susceptible animal. Had the disease appeared, it would have been diagnosed with absolute certainty.

In the above tables the infections are mentioned which appeared in the animals during the course of the experiment. It will be seen that the following infections were diagnosed: Heartwater, *Piroplasma bigeminum*, *Anaplasma marginale*, *Theileria mutans*, *Trypanosoma congolense*, *Trypanosoma vivax* and *Spirochaeta theileri*. There is, therefore, ample corroboration in this experiment, of the results obtained in experiments 5, 6, 7 and 8 above, in which it was attempted to produce a relapse of East Coast fever by infecting immune animals with various diseases. From Table 1 it will be seen that of the 25 immune animals in Experiment 9 more than half showed *Piroplasma bigeminum* in their blood and three probably died of this infection. *Heartwater* was diagnosed in about six animals, and *Anaplasma marginale* in 15. One animal died of an infection with *Tryp. vivax*. In none of these cases was there any suspicion of East Coast fever.

It will further be seen that *Theileria mutans* was diagnosed in very many of the animals. This parasite was recorded in 22 out of the 25 immune, and in 23 out of the 33 susceptible animals. This finding is in accordance with the general experience in South Africa, namely that practically 100 per cent. of the cattle become infected with this ubiquitous, harmless parasite.

The question may be raised at this stage, whether the "small piroplasms" which were always referred to as "*Theileria mutans*" were not, in some cases at least, "*Theileria parva*," and whether some of these cases should not be regarded as mild attacks of East Coast fever. The answer to this question is, in our opinion, emphatically in the negative. It is true that a distinction between the blood forms of these two parasites is practically impossible, but there are other factors which enable us to decide which of the two parasites we are dealing with. Ordinarily the presence or absence of "Koch's bodies" decides whether it is a case of East Coast fever or not; although, as has been recently shown by various authors (c.f. Du Toit, 1930), Koch's bodies may also be present in cases of *Theileria mutans* infection (see below). But more important is the course of the disease and the subsequent history of the infection.

Under South African conditions *Theileria mutans* is always a mild and perfectly innocuous parasite which produces practically no symptoms at all and is never responsible for the death of an animal. This has also been the case in our present experiment. On the other hand, *Theileria parva* is, in our experience, a very deadly parasite which causes a mortality of 90 or 95 per cent. *East Coast fever is a disease which could not escape detection under the conditions of this experiment.* Even if we assume that one or a few of

these animals had mild attacks of East Coast fever, which were wrongly diagnosed as *Theileria mutans* infection, then the real position would still have been disclosed in a comparatively short time. Ticks would have "picked up" the infection on this hypothetical first case and would have carried it to the susceptible cattle, and soon the whole camp would have been ablaze with East Coast fever. Our experience in South Africa of this insidious disease is so great that we can predict with absolute certainty what would have happened if one case of East Coast fever had appeared amongst these animals.

With regard to the occurrence of "*Koch's bodies*" a few observations should be recorded. In a gland smear of cow 1863 a body was seen on the 27th December, 1929, which resembled a "Koch's body" to some extent, and in gland smears of ox 2336 three such bodies were seen on the 26th November, 1928. These bodies most probably represented developmental stages of *Theileria mutans*. Several such cases have been observed recently (see Theiler and Graf, 1928), and the whole matter has been fully discussed by the present author in a report made to the Eleventh International Veterinary Congress in London in 1930. A further case of this nature was observed in ox 2207 on the 6th September, 1928. In this case subinoculations of blood were made into six susceptible calves, but no further Koch's bodies were found. This case has been discussed in the report just referred to.

These three cases were the only ones in which "Koch's bodies" were ever seen. That they cannot be regarded as cases of East Coast fever has been explained above.

All the animals in Experiment 9 were exposed to very great hardships during the three years. This fact is borne out by large percentage of deaths (48 per cent.). It will also be noticed that a fairly large number died of cachexia and inanition. This must be ascribed partly to the poor grazing at various times, and partly to the presence of various infectious diseases and the tremendous *tick infestation*. It was stated at the beginning that one of the reasons why this particular site was selected was the presence of large numbers of ticks. The idea was, of course, that if there should be a case of East Coast fever, there would be enough ticks to transmit it. The accompanying photographs (Figs. 4 to 9) show how excessive the degree of tick infestation was in these animals. Recently it has been found necessary to "hand dress" the ears of these animals in order to give them some relief.

For general interest photographs (Figs. 10 to 13) are added here of animals running in a camp adjacent to our experimental camp, but which were dipped regularly once a week. The complete absence of ticks on these animals and their contented appearance illustrate the great benefit of regular dipping and are in striking contrast with the condition revealed by the above photographs in the experimental animals.

The experiment described above will be closed within a very short time. There seems to be no necessity to continue it any longer. The cattle will be slaughtered and the camp will then be restocked with animals connected with other experimental work. These animals will be treated in the same way as those at present in the experiment, that is, their temperatures will be taken daily and smears will be examined regularly. In this way a further control will be kept on the results of the present experiment. Ticks which have dropped off the present animals will bite the new animals and, if there is any East Coast fever present, the infection will appear in the new animals and will be be diagnosed. It is proposed to continue this arrangement for at least another year.

Summary and Conclusions.

1. Experiments are described in this paper which were undertaken with the object of determining whether an animal which has recovered from East Coast fever can get a relapse and thus be responsible for a new outbreak of the disease.

2. Attempts were made ot produce a relapse by injecting "provocatives" (sterile milk, egg albumen, trypanblue); by producing *anaemia* (with the aid of drugs or by withdrawing large quantities of blood); by *removing the spleen*; by inflicting *bodily injuries* (fractures); by producing *peritonitis*; by producing *abortion* and *metritis*; by infecting recovered animals with *diseases* (trypanosomiasis, heartwater).

All these attempts failed. A relapse never appeared, neither in a clinical sense, nor could the parasites of East Coast fever ever be diagnosed microscopically.

3. It was concluded from these experiments that it did not seem possible to produce a relapse in an animal which had recovered from East Coast fever; and that such an immune animal did not appear to be a source of danger. Hence it would appear to be perfectly safe to move an immune animal into a clean area, since it could not start a fresh infection in the absence of infected ticks.

4. Further corroboration of this conclusion was afforded by an experiment which was carried out under natural and extremely rigorous conditions. Recovered (immune) and susceptible cattle were run together in a camp which was heavily infested with ticks (including the transmitters of East Coast fever) for three years. Various infectious diseases appeared amongst the animals and nearly half of them died, but there was never a suspicion of East Coast fever.

5. These experiments seem to have exonerated the immune ox from all blame in connection with new outbreaks of East Coast fever. In such outbreaks the source of infection must be looked for in newly introduced infected cattle or infected ticks.

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Only the few articles referred to in this paper are quoted below:—

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FIG. 1.—Veld in camp at Kaalplaas.

FIG. 2.—Camp with hill in background showing covering of trees (mostly Mimosas).

FIG. 3.—View towards north showing two parallel boundary fences of camp.

FIGS. 4 and 5.—Tick-infested and emaciated cattle in experimental camp.

FIG. 6.—Extreme degree of tick infestation on ear, face and eyelids of calf.

FIG. 7.—Ear of animal in experimental camp covered with ticks.

FIG. 8.—Chest and front legs of animal showing tick infestation.

FIG. 9.—Udder and adjacent parts of cow showing tick infestation.

FIGS. 10 and 11.—Animals in camp adjacent to experimental camp showing complete freedom of ticks owing to regular weekly dipping.

FIGS. 12 and 13.—Animals in camp adjacent to experimental camp showing complete freedom of ticks owing to regular weekly dipping.

Treatment of Piroplasmoses with T.21.

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

Through the courtesy of the "Chemische Fabrik vorm. Sandoz," Basel, there has been made available, during some years, a series of preparations for use chiefly in the treatment of piroplasmoses of domestic animals. Recently Theiler (1930) has issued a report on two of these preparations, namely, T.18 and T.19. As he explains in his articles, T.18 and T.19 are preparations consisting of Trypan blue combined with sodium cholate.

This article deals with cases of piroplasmosis of the bovine and the dog and of nuttaliosis of the horse treated with a further member of the series, namely, T.21. No information *re* the composition of this member of the series is available.

In short, it may be said that the treatment of bovine piroplasmosis has been very satisfactory, of equine nuttaliosis very promising and of canine piroplasmosis satisfactory, although it does not, in the last-mentioned disease, appear that this drug has any marked superiority over Trypan blue.

The administration of the drug in cattle, horses and dogs, has at no time, produced any ill-effects. In this respect it may perhaps claim definite advantage over Trypan blue, especially when doses somewhat on the large side are used.

The treatment of each of the three diseases mentioned with T.21 is dealt with under each species. In every case the T.21 was given intravenously in saline, except in the cases mentioned in the dog when it was given subcutaneously. The strength of the solutions was either 1 per cent. or 1.5 per cent.

Bovine Piroplasmosis.

No arranged experiment was entered into to test out the efficacy of T.21 in piroplasmosis caused by *Piroplasma bigeminum*. But from time to time cattle which were under observation for other purposes became available for treatment, the effects of which could be judged by the temperature curve and blood-smear examination. The details of the few controlled cases are arranged on the temperature charts, and the reading of the effect of the drug in each case is thus facilitated. For this purpose Charts I to IV are submitted.

CHART I.

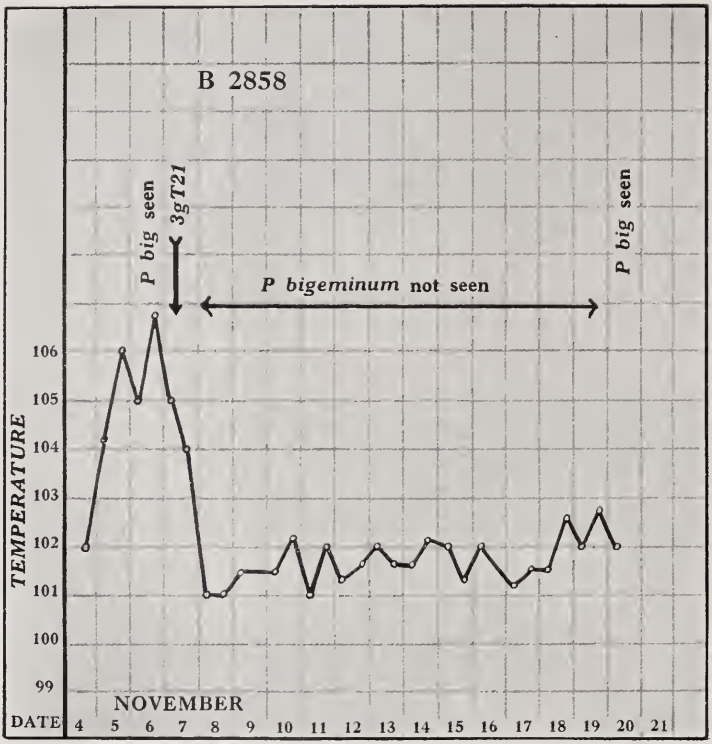

B 2858
P big seen
3gT21
P bigeminum not seen
P big seen
TEMPERATURE
106
105
104
103
102
101
100
99
NOVEMBER
DATE
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

CHART II.

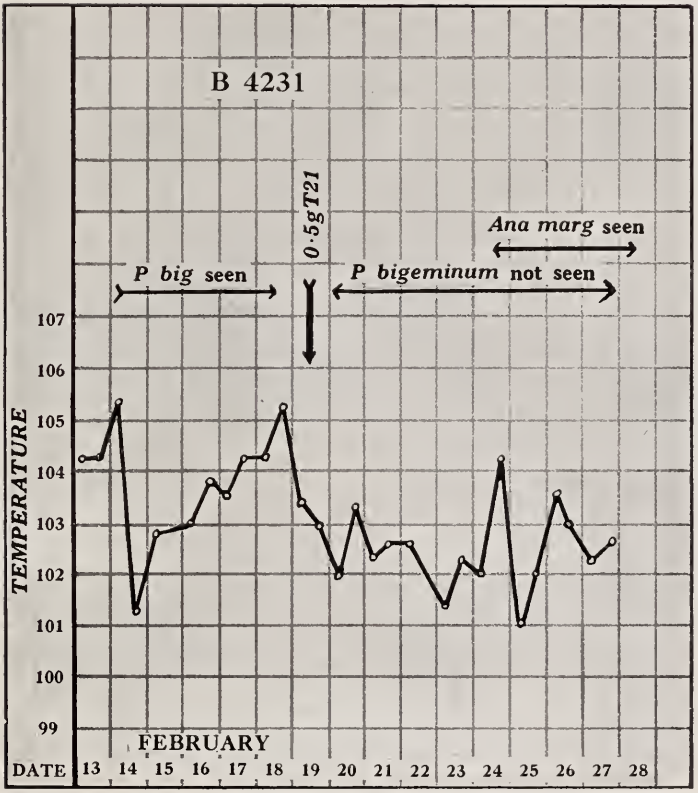

B 4231
0·5gT21
Ana marg seen
P big seen
P bigeminum not seen
TEMPERATURE
107
106
105
104
103
102
101
100
99
FEBRUARY
DATE
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

CHART III.

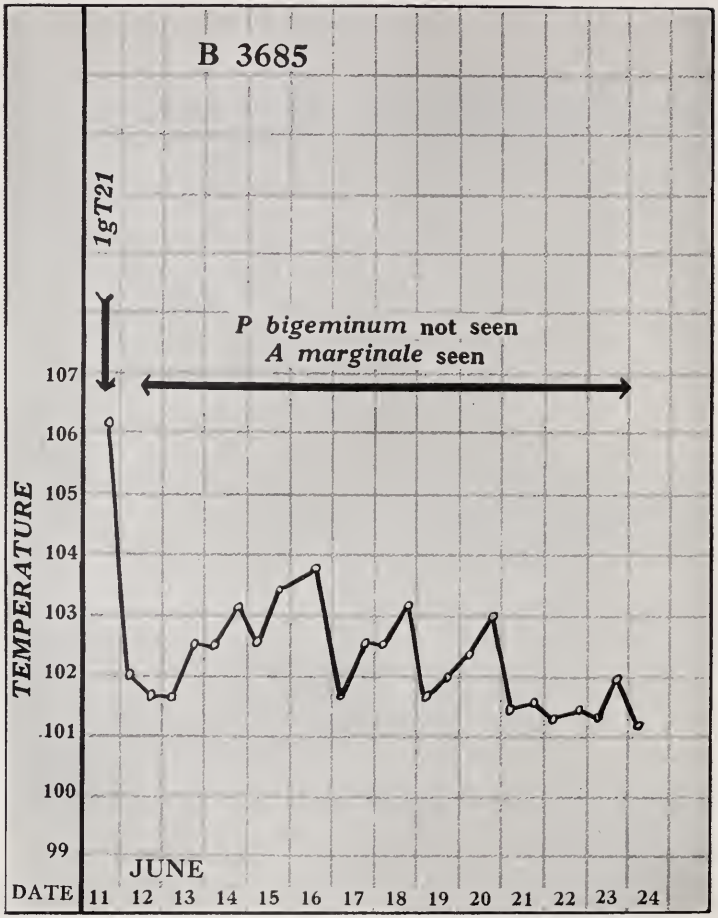
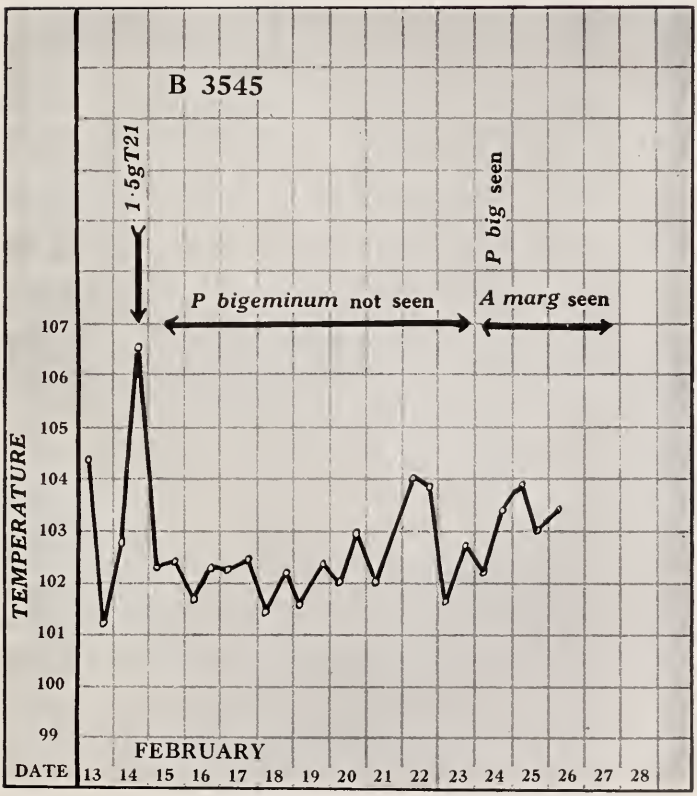

B 3685
1gT21
P bigeminum not seen
A marginale seen
TEMPERATURE
107
106
105
104
103
102
101
100
99
DATE
JUNE
11 12 13 14 15 16 17 18 19 20 21 22 23 24

CHART IV.


B 3545
1·5gT21
P bigeminum not seen
P big seen
A marg seen
TEMPERATURE
107
106
105
104
103
102
101
100
99
FEBRUARY
DATE
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

No endeavour was made to obtain information, in the treatment of these four bovines, of the minimal effective dose of T.21. As the animals were in experiments planned for other purposes, the main object of treatment was to obtain quick recoveries. The bovines 2858 and 3545 were both adults, the former being an imported pure-bred Hereford. The other two were approximately 6 and 9 months old and were well developed. In other cases of piroplasmosis which were not on regular temperature and blood-smear examinations as satisfactory results were obtained by the use of smaller doses.

Three of the bovines, as is illustrated by the charts, showed the presence of *Anaplasma marginale* soon after and, in one case, actually at the time of the injection. No effect on the anaplasmata was observed.

It will be noted also that *P. bigeminum* was subsequently found in blood smears in two of the cases, namely 2858 and 3545. The drug thus did not result in sterilization of the bovines.

In every case a normal temperature was obtained within 24 hours of the injection, a state which corresponded to a general improvement of the animals.

Equine Nuttalliosis.

The subjects for treatment with T.21 were obtained by the sub-inoculation of blood from horse 19683 in a blood smear of which *Nuttallia equi* was found. The horses were obtained from a supposed tick-free area. However, of the six horses sub-inoculated, only three showed *Nuttallia equi* in their blood smears. It is the treatment of two of these three horses, the third being kept as a control, that is described hereunder.

The charts herewith are of the control and of the two treated cases, one of which was treated somewhat earlier in the reaction than the other. In both the treated cases the dose of T.21 was 1.5 gm. in 100 c.c. saline and the solution was given intravenously. The arrows on the charts indicate the date of injections of the T.21.

CHART V.

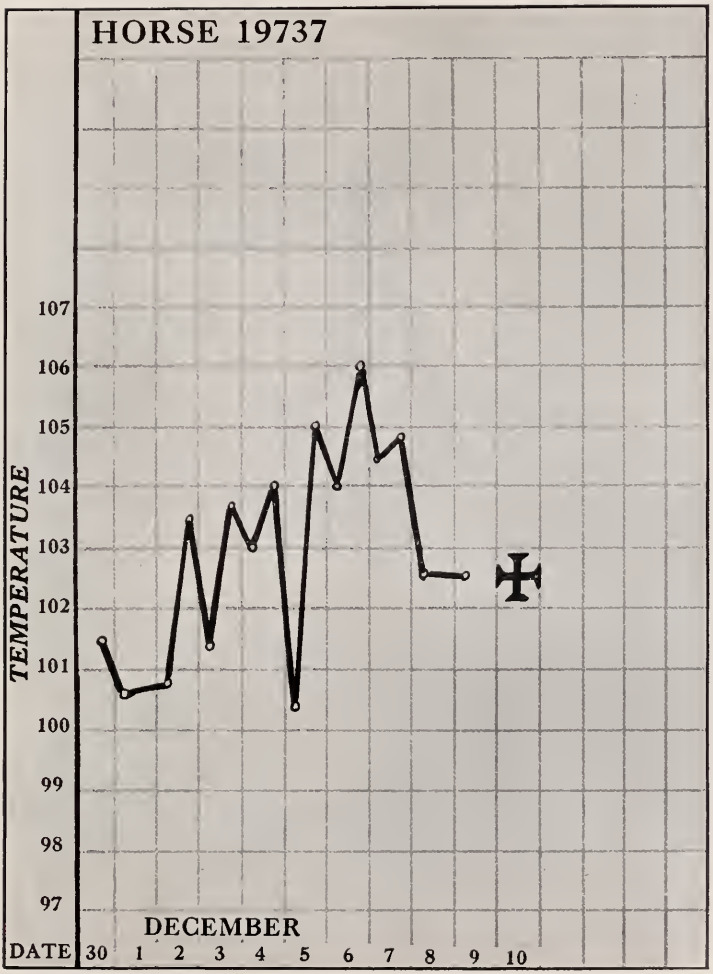

HORSE 19737
TEMPERATURE
107
106
105
104
103
102
101
100
99
98
97
DECEMBER
DATE
30 1 2 3 4 5 6 7 8 9 10

CHART VI (Horse 19733).

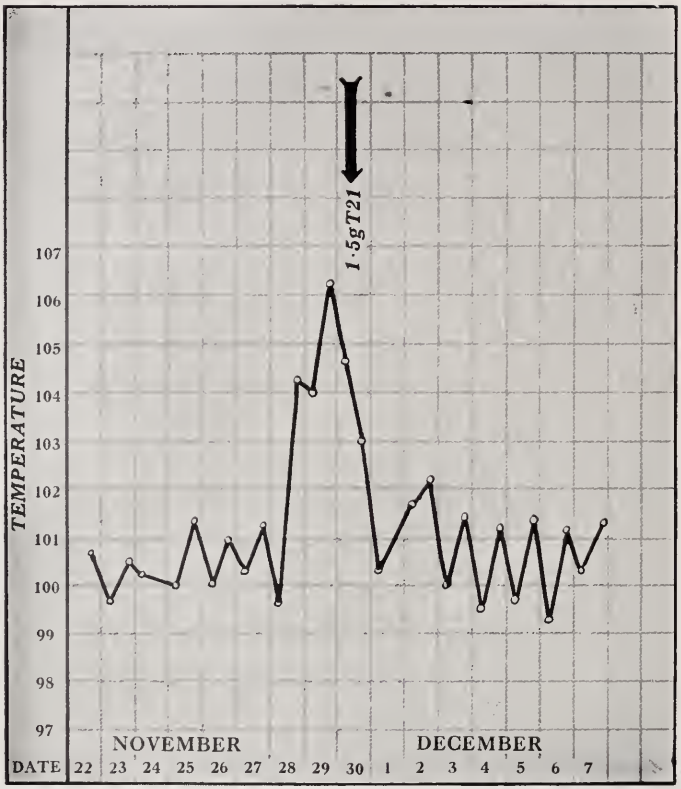
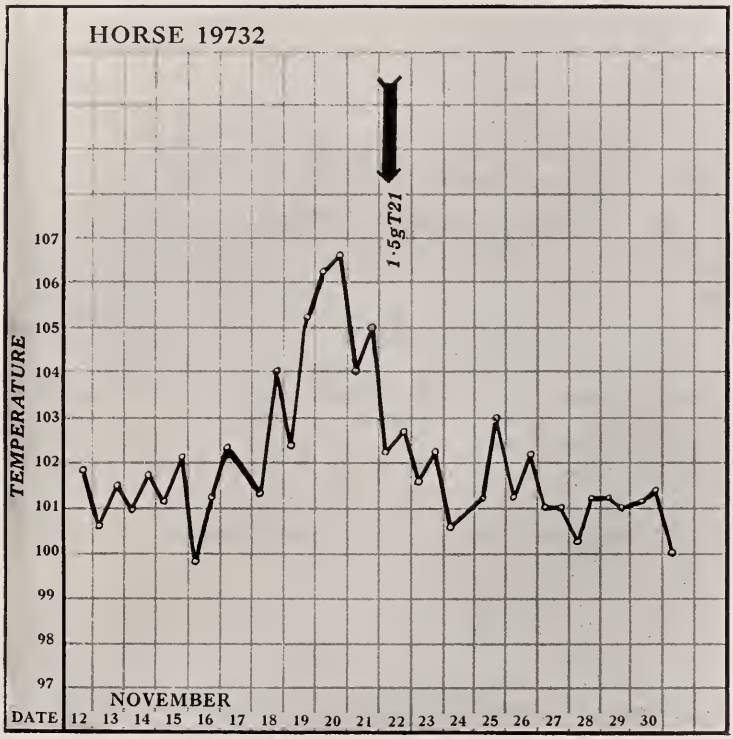


CHART VII.


HORSE 19732
1·5gT21
TEMPERATURE
107
106
105
104
103
102
101
100
99
98
97
NOVEMBER
DATE
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

In addition to the two cases treated above, an opportunity was afforded of treating a heavy imported hunter which contracted acute nuttalliosis, the blood smear showing frequent parasites. This animal was given 2 gm. of T.21 intravenously in solution and made an uncomplicated recovery.

Discussion.

The first two cases, the 1st and 2nd generations of the sub-inoculations, were treated with good results, as can be seen from examination of Charts VI and VII. Horse 19737, which was in the 3rd generation, and which did not at the time appear to be suffering from as severe an attack as the two selected for treatment, was kept as control.

The dose used in both cases was the same and could not be regarded as being on the large size. It was given in 100 c.c. saline. Yet the result was immediate and lasting, for relapses did not occur, although in one case *Nuttallia equi* was found subsequently.

The pathological anatomical findings of the control horse were briefly as follows: General icterus; marked enlargement of the spleen; petechiae ecchymoses and extravasations of right and left endocardium and epicardium; multiple haemorrhages of kidney and gastro-intestinal tract; degenerative changes of myocardium, kidney and liver; emphysema of lungs; pigmentation of liver.

It should, however, be borne in mind that, notwithstanding that the control horse, judging from the temperature curve alone, was apparently not subjected to as severe an attack as the other two, yet there might have been produced an increased virulency of the parasite by passage. The control was in the third generation and sub-inoculations were in every case made at the height of the reaction, i.e. just before the injection of T.21. The blood-smear examination showed *Nuttallia equi* to be more frequent in the control than in the others.

Canine Piroplasmosis.

A number of cases of canine piroplasmosis have been treated with T.21. As the majority of cases treated was in out-patients, in only a proportion of the cases was any information as to the outcome obtained, for after the injection the out-patients are returned to the owners. If, however, no improvement resulted or if a dog subsequently relapsed, it may be taken that the owner would, in most cases, bring the dog back. Judging entirely on the number of dogs which were brought back after treatment, one can place the number of relapses after treatment with T.21 as approximately 50 per cent. It should be noted, however, that the dose of T.21 used was in most cases less than the dose of Trypan blue which would have been used under similar circumstances. This policy was undertaken deliberately for the purpose of determining whether T.21 had a marked superiority over Trypan blue in the treatment of canine piroplasmosis.

A few examples of the treatment of canine piroplasmosis in out-patients are the following:—

(1) Fox-terrier of P. J. J. Clinical piroplasmosis confirmed by blood-smear examination. It was given 5 c.c. of 1.5 per cent. solution of T.21. It showed a relapse on the 8th day. It was then given 12 c.c. of 1.5 per cent. solution of Trypan blue. Recovery.

(2) Wire-hair terrier of V. S. Clinical piroplasmosis confirmed by blood-smear examination. It was given 5 c.c. of 1.5 per cent. solution of T.21. It relapsed on 21st day and was given 7.5 c.c. of 1.5 solution of Trypan blue. It again relapsed on the 10th day and was given 15 c.c. of 1.5 per cent. solution of Trypan blue. Recovery.

(3) Irish Setter of T. This was a very acute case of piroplasmosis associated with acute ulcerative stomatitis and pharyngitis. It was given 10 c.c. of 1.5 per cent. solution of T.21. Improvement was marked in 3 hours. By the following day the stomatitis and pharyngitis had greatly improved. The dog was discharged on the 7th day as recovered.

It must be noted here that relapses after the treatment of piroplasmosis of the dog with Trypan blue are not at all uncommon. The strain of *P. canis* met with in this area is more often than not ex-extremely virulent. This virulency seems to be associated with a poor response to treatment to doses of Trypan blue which, in other areas, might be ample to produce complete recovery. The author has had the opportunity of treating in another area with Trypan blue a large number of cases of canine piroplasmosis. The results with approximately half the dose used here were good and in not a single case was a relapse met with.

Cases which relapsed after a T.21 injection were usually given as a second injection Trypan blue. The dose always, however, was a larger one. No interference with the efficacy of the latter injection was noted as a result of the use of the former. But in some cases good results were also obtained in relapses after Trypan blue injections by the use of T.21 as the second injection. No decreased efficiency of either Trypan blue or T.21 has been noted, even when it has been necessary to give a third injection. This is somewhat contrary to the formerly held opinion of the treatment of canine piroplasmosis with Trypan blue.

During an experiment planned for a different purpose an opportunity was afforded of treating a number of pups infected with an extremely virulent local strain of *P. canis*. Table I gives the details of the various generations and indicates, by means of blood-smear examinations and the length of the course of the disease, the marked virulency of the strain. Table II gives the details of those few cases which were treated, and Charts VIII-XI are submitted to illustrate the acuteness of some cases, and the effects of early and late

treatment. On account of the requirements of the main experiment, treatment in some cases could only be instituted when the animals were *in extremis*:—

TABLE I.

Generation.	Nos. of Dogs.	*P. canis* Found on	Died on.	Remarks.
1st	775	7th day	9th	—
	777	—	12th	—
2nd	781	5th day	6th	—
	782	5th day	7th	—
3rd	783	—	4th	—
	784	5th day	5th	Treated. See Table II.
4th	776	2nd day	4th	,, ,,
	785	3rd day	—	,, ,,
5th	779	3rd day	6th	—
	780	6th day	—	Treated. See Table II.
6th	816	4th day	—	,, ,,
	817	3rd day	4th	—
7th	814	4th day	8th	,, ,,
	818	2nd day	—	,, ,,

Discussion.

Seven of the dogs were not given treatment. All of these died. The appearance of the parasite was remarkably early. In the case of dog 818 the sub-inoculation from dog 816 was carried out at 9 a.m. on 23.2.30 and *P. canis* was recorded as very rare in the blood smear made at 2.30 p.m. on 25.2.30.

CHARTS VIII AND IX.

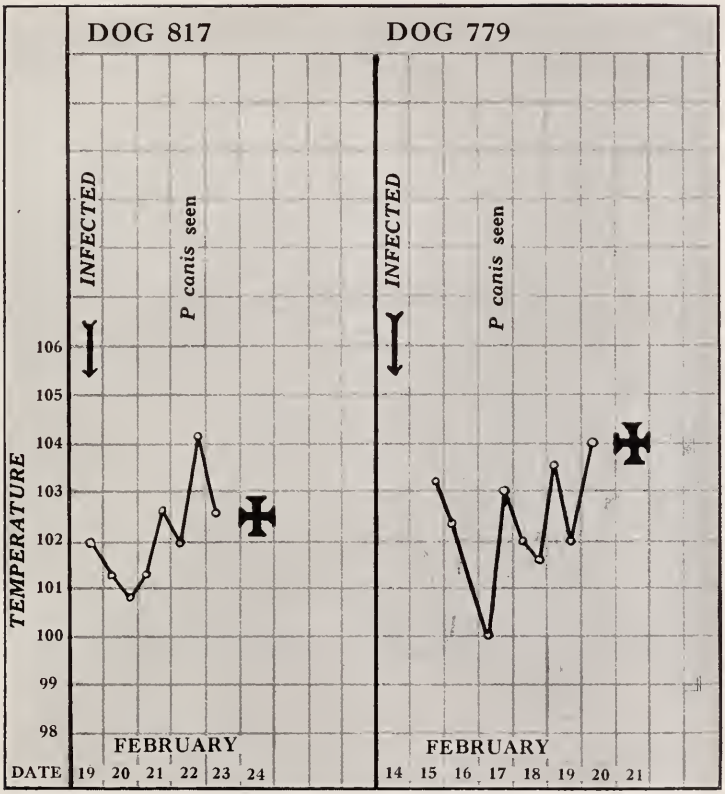


CHART X.

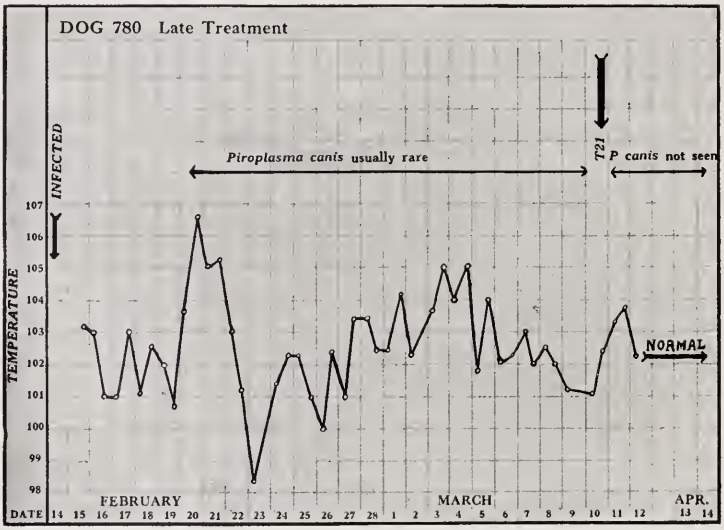

DOG 780 Late Treatment
INFECTED
T21
Piroplasma canis usually rare
P canis not seen
NORMAL
TEMPERATURE
107
106
105
104
103
102
101
100
99
98
DATE
FEBRUARY
MARCH
APR.
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14

CHART XI.

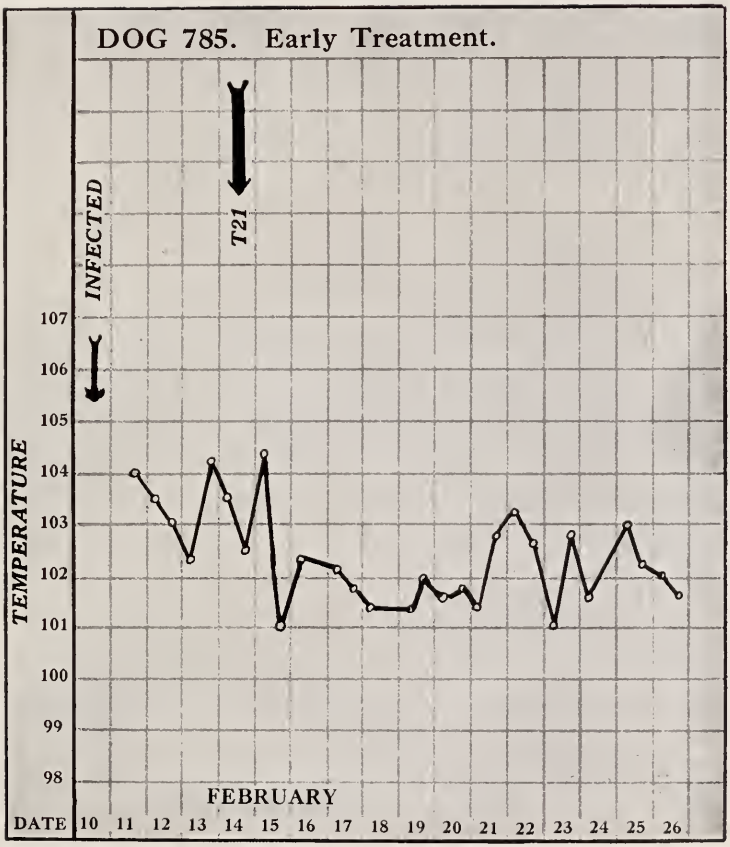

DOG 785. Early Treatment.
INFECTED
T21
TEMPERATURE
107
106
105
104
103
102
101
100
99
98
FEBRUARY
DATE
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

TABLE II.

Treatment of Canine Piroplasmosis.

Nos.	Weight in Kg.	Drug.	Dose.	Remarks.
784	2·7	T.21	2·5 c.c. of 1·5% sol.	Dog was *in extremis* when treated. It died on the same day.
776	3·2	T.B.	5 c.c. of 1·5% sol.	Dog was *in extremis* when treated. It died on evening of same day.
785	3·6	T.21	5 c.c. of 1·5% sol.	Recovery.
780	3·7	T.21	3 c.c. of 1·5% sol.	Recovery.
816	3·2	T.21	3 c.c. of 1·5% sol.	Recovery.
814	3·4	T.21	2 c.c. of 1·5% sol.	Dog was *in extremis* when treated. It died on same day.
818	2·4	T.21	3 c.c. of 1·5% sol.	Relapse on 24th day.
—	—	T.21	3 c.c. of 1·5% sol.	Given on 32nd day. *P. canis* found on 33rd day. It died on 35th day.

Discussion.

Of the seven dogs which were treated three recovered, three died on same day as treatment was carried out and one relapsed and died on 35th day after infection. Two of the recoveries are illustrated by Charts X and XI. The dose of T.21, which was approximately .012 to .02 gm. per Kg. of body weight, was sufficient to bring about a recovery or control the disease in those which were not *in extremis* when treated. Two of the dogs, viz., 780 and 814 were given T.21 subcutaneously. It is difficult to draw any conclusions from the results, but if Trypan blue had been used in place of the T.21 as good results would have been expected. A disappointing result was that of dog 818. The report of this one is given in somewhat greater detail below.

D. 818. Bitch aged six weeks. Infected by injection of 3 c.c. blood of dog 816 on 23.2.30. *P. canis* was found on 25.2.30, becoming very frequent of 27.2.30, when it was treated with T.21. The temperature subsided gradually during the next two days. Daily smears were examined, and it was not until 19.3.30 that *P. canis* was found, becoming very frequent on 27.3.30, when it was again injected with T.21. The dog died on 29.3.30. During the whole course of the disease the dog was dull, did not feed satisfactorily and the mucous membranes remained pale. The pulse was weak and averaged approximately 120 per minute.

Summary.

1. Doses of T.21 varying from 0.5 to 3 gm. were in every case of bovine piroplasmosis efficacious.

2. Three cases of equine nuttalliosis treated with T.21 in doses of 1.5 to 2 gm. recovered in a most satisfactory manner.

3. The treatment of canine piroplasmosis with T.21 did not show any marked superiority over that with Trypan blue.

4. Failure to treatment with either T.21 or Trypan blue did not apparently interfere with subsequent treatment with either one or the other of these two drugs.

REFERENCES.

THEILER, A. (1930). Un nouveau médicament pour le traitement des piroplasmoses. *Bull. Soc. Path. Exot.*, 1930, 14th May, Vol. 23, No. 5, pp. 506-529.

Blood Parasites of Game in Zululand.

PRELIMINARY REPORT.

By W. O. NEITZ, B.V.Sc., Veterinary Research Officer, Onderstepoort.

The intention of this paper is to place on record a list of blood parasites found in game shot in the Hlabisa and Lower Umfolozi magisterial districts, of Zululand. The Director of Tsetse-Fly Control Operations, Mr. R. H. T. P. Harris and his staff were kind enough to send blood, spleen and lymph gland smears from various wild animals to this Institute.

The chief object for examining these smears was to find out to what extent game living in the abovementioned tsetse-fly areas harbour pathogenic trypanosomes. Two blood, two spleen and two gland smears were examined from each of four hundred and forty animals and these results will be given below. The idea of shooting the game in the abovementioned districts was to create buffer zones between the game reserves and the stock farms (Harris 1930). It was found that by destroying the game the number of tsetse-flies decreased considerably.

The examination showed that trypanosomes could be demonstrated only in four animals, namely in two bushbuck, one kudu and one zebra. In one bushbuck the trypanosomes were identified as *T. virax* and in the remaining three animals as *T. congolense.*

The occurrence of trypanosomes in game in Zululand has been demonstrated by Bruce, Mitchell and Curson. In the following table details regarding game trypanosomes from Zululand are appended.

TABLE I.

Game Animal (Host).	Type of Trypanosoma.	Demonstrated by.	Year.	Publication.
Kudu (3 cases)..	Apparently *T. brucei*	Bruce.....	1895	Further Report on Tsete fly.
Buffalo.....	,, ,,	,, ...	,,	Disease in Zululand.
Bushbuck.....	,, ,,	,, ...	,,	,, ,,
Wildebeest (3)....	,, ,,	,, ...	,,	,, ,,
Hyaena.....	,, ,,	,, ...	,,	,, ,,
Reedbuck.....	,, ,,	,, ...	1903	Appendix to further report.
Kudu.....	,, ,,	,, ...	,,	,, ,,
Steenbuck.....	,, ,,	,, ...	,,	,, ,,
Impala.....	*T.*.....	Mitchell.....	1921	Tvl. Museum. Zululand Expedition, 1914.
Bushbuck.....	*T.*.....	,, ...	,,	,, ,,
Zebra.....	*T.*.....	,, ...	,,	,, ,,
Kudu.....	*T.*.....	,, ...	,,	13th and 14th Report of the Director of Veterinary Education & Research, Part I, 1928
Duiker.....	*T. vivax*.....	Curson.....	1921	
Warthog.....	*T. vivax*.....	,, ...	,,	

An interesting observation was made in connection with members of the Family *Babesidae* and *Theileridae* in various animals. Small piroplasms resembling *Theileria mutans* have been described from game animals from all over the world especially East and Central Africa. In South Africa, however, these parasites have been described only in a few cases. Theiler (1909) demonstrated the presence of *Nuttalia equi* in the zebra, and also found Koch's bodies in spleen smears from a bushbuck. Subsequently Martinaglia (1930) found *Babesia* in blood from a sable antelope that had died in the Zoological Gardens, Johannesburg. Quite a number of wild animals in the present study were found infected. In some the infection was very light and in others there were numerous parasites present.

The significance of these parasites is not fully known. What part do they play in the animal? Can they be transmitted from one member of a sub-family to another? The majority of the *Babesidae* are specific for a particular species of animal, but successful transmission was possible with (*a*) *Piroplasma bigeminum* which can be transmitted to the buffalo and the white tailed deer (*Odocoilens chiriquensis*) from Panama; (*b*) *N. equi* which has been demonstrated in the zebra; (*c*) *Babesia gibsoni* that can be transmitted from the jackal to the dog; (*d*) *Theileria ovis* which is transmissible to the goat, and (*e*) *Theileria recondita* which is transmissible to the goat.

The following tables represent the results of the examination of game smears from Hlabisa and Lower Umfolozi districts. For convenience the results of the Lower Umfolozi district are sub-divided into (i) Empangeni, and (ii) Umfolozi River. A plus sign means that parasites were found, a minus sign that no parasites were found, and a circle that the smear was badly prepared.

Table II.—List of wild animals from Hlabisa showing parasites.

Table III.—List of wild animals from Empangeni showing parasites.

Table IV.—List of wild animals from Umfolozi River showing parasites.

TABLE II.—HLABISA.

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
6/6/28	Zebra.....	25	+	−	−	−	−	−	−	−	−	
6/6/28	,, ...	29	+	−	−	−	−	−	−	−	−	
6/6/28	,, ...	61	+	−	−	−	−	−	−	−	−	
18/6/29	,, ...	65	+	+	−	−	−	−	−	−	−	
24/6/29	,, ...	104	+	−	−	−	−	−	−	−	−	
8/7/29	,, ...	165	+	−	−	−	−	−	−	−	−	
8/7/29	,, ...	168	+	−	−	−	−	−	−	−	−	
8/7/29	,, ...	169	+	−	−	−	−	−	−	−	−	
8/7/29	,, ...	170	+	+	−	−	−	−	−	−	−	
8/7/29	,, ...	180	+	+	+	−	−	−	−	−	−	
13/7/29	,, ...	195	+	○	○	−	−	−	−	−	−	
13/7/29	,, ...	197	+	−	−	+	−	−	−	−	−	*T. congolense.*
13/7/29	,, ...	214	+	○	+	−	−	−	−	−	−	
15/7/29	,, ...	216	+	−	−	−	−	−	−	−	−	
22/7/29	,, ...	236	+	−	−	−	−	−	−	−	−	
17/8/29	,, ...	311	+	+	○	−	−	−	−	−	−	
6/6/29	Bushbuck...	13	+	+	−	−	−	−	−	−	−	
6/6/29	,, ..	26	+	−	−	−	−	−	−	−	−	
6/6/29	,, ..	44	+	−	−	−	−	−	−	−	−	
19/6/29	,, ..	109	+	−	−	−	−	−	−	−	−	
24/6/29	,, ..	110	+	−	−	−	−	−	−	−	−	
13/7/29	,, ..	198	+	+	+	−	−	−	−	−	−	
13/7/29	,, ..	208	+	−	−	−	−	−	−	−	−	
16/7/29	,, ..	219	+	+	+	−	−	−	−	−	−	Koch's bodies in spleen.
16/7/29	,, ..	221	+	+	+	−	−	−	−	−	−	
17/8/29	,, ..	308	+	+	+	−	−	−	−	−	−	
17/8/29	,, ..	314	+	+	−	−	−	−	−	−	−	
17/8/29	,, ..	315	+	+	−	−	−	−	−	−	−	
6/7/29	Duiker.....	161	+	−	○	−	−	−	−	−	−	
22/7/29	,, ...	228	+	−	−	−	−	−	−	−	−	
8/7/29	Mountain Reedbuck	193	○	+	○	−	−	−	−	−	−	
6/8/29	,,	297	+	−	−	−	−	−	−	−	−	
17/8/29	,, ...	312	+	+	+	−	−	−	−	−	−	
17/8/29	,, ...	317	+	−	−	−	−	−	−	−	−	
6/6/29	Waterbuck..	10	+	+	−	−	−	−	−	−	−	
6/6/29	,, .	31	+	−	−	−	−	−	−	−	−	
15/6/29	,, .	56	+	−	−	−	−	−	−	−	−	
8/7/29	,, .	176	+	+	−	−	−	−	−	−	−	
8/7/29	,, .	177	+	−	−	−	−	−	−	−	−	
26/7/29	,, .	273	+	−	−	−	−	−	−	−	−	
15/6/29	Kudu.....	68	−	−	−	+	−	−	−	−	−	*T. congolense.*
8/7/29	Blue Wildebeest	167	+	+	+	−	−	−	−	−	−	
8/7/29	,, ...	174	+	+	−	−	−	−	−	−	−	
8/7/29	,,	181	○	+	+	−	−	−	−	−	−	
6/8/29	Steenbuck..	296	+	+	−	−	−	−	−	−	−	
6/7/29	Reedbuck...	159	+	−	−	−	−	−	−	−	−	
9/7/29	,, ..	194	+	+	−	−	−	−	−	−	−	
15/7/31	,, ..	217	+	+	−	−	−	−	−	−	−	

TABLE II.—HLABISA—*Continued.*

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
6/8/29	Reedbuck ..	291	+	—	○	—	—	—	—	—	—	
6/8/29	,, ..	316	+	—	—	—	—	—	—	—	—	
26/7/29	,, ..	261	+	+	—	—	—	—	—	—	—	
26/7/29	,, ..	263	+	—	—	—	—	—	—	—	—	
26/7/29	,, ..	264	+	—	—	—	—	—	—	—	—	
26/7/29	,, ..	268	+	—	—	—	—	—	—	—	—	
17/8/29	,, ..	299	+	+	—	—	—	—	—	—	—	
17/8/31	,, ..	318	+	—	—	—	—	—	—	—	—	
17/8/29	,, ..	319	+	—	—	—	—	—	—	—	—	
17/8/29	,, ..	328	+	+	—	—	—	—	—	—	—	

TABLE III.—EMPANGENI.

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
19/6/29	Zebra.....	76	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	80	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	81	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	84	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	85	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	86	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	89	+	—	—	—	—	—	—	—	—	
19/6/29	,, ...	90	+	—	—	—	—	—	—	—	—	
28/6/29	,, ...	145	+	—	—	—	—	—	—	—	—	
28/6/29	,, ...	147	+	+	—	—	—	—	—	—	—	
9/7/29	,, ...	186	+	+	+	—	—	—	—	—	—	
9/7/29	,, ...	187	○	+	—	—	—	—	—	—	—	
9/7/29	,, ...	188	+	—	—	—	—	—	—	—	—	
9/7/29	,, ...	189	+	—	—	—	—	—	—	—	—	
9/7/29	,, ...	191	+	○	+	—	—	—	—	—	—	
23/7/29	,, ...	245	+	—	—	—	—	—	—	—	—	
6/8/29	,, ...	278	+	—	—	—	—	—	—	—	—	
17/8/29	,, ...	304	+	○	○	—	—	—	—	—	—	
19/6/29	Bushbuck...	97	—	+	+	—	—	—	—	—	—	
19/6/29	,, ..	98	+	+	—	+	—	—	—	—	—	*T. Congolense.*
20/6/29	,, ..	139	+	—	—	—	—	—	—	—	—	
23/7/29	,, ..	246	+	+	—	—	—	—	—	—	—	
23/7/29	,, ..	247	+	—	—	—	—	—	—	—	—	
9/16/29	Duiker.....	88	+	○	—	—	—	—	—	—	—	
19/6/29	,, ...	93	+	+	—	—	—	—	—	—	—	
9/7/29	,, ...	185	—	+	—	—	—	—	—	—	—	
9/7/29	,, ...	195	+	+	+	—	—	—	—	—	—	
23/7/29	,, ...	244	+	—	—	—	—	—	—	—	—	
6/8/29	,, ...	277	+	—	—	—	—	—	—	—	—	
19/6/29	Waterbuck..	100	+	+	+	—	—	—	—	—	—	
26/7/29	Antbear....	259	+	+	—	—	—	—	—	—	—	

TABLE IV.—UMFOLOZI RIVER.

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
22/10/29	Zebra.....	347	+	+	+	−	−	−	−	−	−	
22/10/29	,, ...	371	+	−	−	−	−	−	−	−	−	
22/10/29	,, ...	373	+	−	−	−	−	−	−	−	−	
22/10/29	,, ...	379	+	−	−	−	−	−	−	−	−	
22/10/29	,, ...	385	+	+	−	−	−	−	−	−	−	
22/10/29	,, ...	390	+	+	+	−	−	−	−	−	−	
21/10/29	,, ...	426	+	+	○	−	−	−	+	−	−	
17/8/29	Bushbuck...	362	+	+	○	−	−	−	−	−	−	
22/10/29	,, ..	364	+	+	+	−	−	−	−	−	−	
22/10/29	,, ..	382	+	−	○	−	−	−	−	−	−	
22/10/29	,, ..	386	+	+	+	−	−	−	−	−	−	
22/10/29	,, ..	394	+	+	−	−	−	−	−	−	−	
29/10/29	,, ..	403	+	+	+	−	−	−	−	−	−	
29/10/29	,, ..	410	+	+	+	−	−	−	−	−	−	
29/10/29	,, ..	422	+	−	−	−	−	−	−	−	−	
29/11/29	,, ..	428	−	−	−	+	+	○	−	−	−	*T. vivax.*
29/10/29	,, ..	434	+	−	−	−	−	−	−	−	−	
29/10/29	,, ..	440	+	+	−	−	−	−	−	−	−	
29/10/29	Duiker.....	349	+	+	○	−	−	−	−	−	−	
29/10/29	,, ...	354	+	−	−	−	−	−	−	−	−	
29/10/29	,, ...	365	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	372	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	378	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	383	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	389	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	392	+	+	−	−	−	−	−	−	−	
29/10/29	,, ...	395	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	398	+	+	○	−	−	−	−	−	−	
29/10/29	,, ...	400	+	+	○	−	−	−	−	−	−	
29/10/29	,, ...	404	+	+	+	−	−	−	−	−	−	
29/10/29	,, ...	411	+	+	○	−	−	−	−	−	−	
29/10/29	,, ...	413	+	+	○	−	−	−	−	−	−	
29/10/29	,, ...	417	+	+	○	−	−	−	−	−	−	
22/10/29	,, ...	421	+	−	−	−	−	−	−	−	−	
22/10/29	,, ...	427	+	+	+	−	−	−	−	−	−	
22/10/29	,, ...	432	+	+	○	−	−	−	−	−	−	
22/10/29	,, ...	437	+	+	○	−	−	−	−	−	−	
22/10/29	Waterbuck..	360	+	+	+	−	−	−	−	−	−	
22/10/29	,, .	370	+	+	+	−	−	−	−	−	−	
22/10/29	,, .	401	+	+	+	−	−	−	−	−	−	
22/10/29	,, .	402	+	+	+	−	−	−	−	−	−	
22/10/29	Kudu.....	332	+	−	−	−	−	−	−	−	−	
22/10/29	,, ...	357	+	+	+	−	−	−	−	−	−	
22/10/29	,, ...	407	+	+	+	−	−	−	−	−	−	
22/10/29	Blue Wildebeest	358	+	+	−	−	−	−	−	−	−	
22/10/29	,,	430	+	+	−	−	−	−	−	−	−	
22/10/29	Reedbuck...	353	+	+	+	−	−	−	−	−	−	
22/10/29	,, ..	435	+	+	+	−	−	−	−	−	−	
22/10/29	Warthog....	391	−	−	−	−	−	−	−	+	−	
29/10/29	,, ...	415	−	−	−	−	−	−	+	−	+	
29/10/29	,, ...	418	−	−	−	−	−	−	+	−	−	
29/10/29	,, ...	524	−	−	−	−	−	−	+	−	−	
29/10/29	,, ...	436	−	−	−	−	−	−	+	+	−	

Details regarding the species of animals, total number examined, the number showing either small piroplasma, trypanosoma or microfilaria are given in the subjoined Table V.

TABLE V.

Animal.	Total Number Examined.	Number found infected with Small Piroplasms.	Number found infected with Trypanosoma.	Number found infected with Microfilaria.
Zebra	127	41	1	—
Bushbuck	60	28	2	1
Duiker	50	27	—	—
Red Duiker	1	—	—	—
Reedbuck	49	15	—	—
Mountain Reedbuck	18	4	—	—
Kudu	8	3	1	—
Warthog	56	—	—	5
Steenbuck	2	1	—	—
Klipspringer	1	—	—	—
Blue Wildebeest	40	5	—	—
Wildcat	1	—	—	—
Otter	1	—	—	—
Antbear	1	1	—	—
Inyala	1	—	—	—
Crocodile	1	—	—	—
Waterbuck	23	11	—	—

Information regarding the species of animals, total number examined from Hlabisa, the number showing either small piroplasma, trypanosoma or microfilaria is given in the following Table VI.

TABLE VI.

Game Animal.	Total Number Examined.	Number found infected with Small Piroplasms.	Number found infected with Trypanosoma.	Number found infected with Microfilaria.
Zebra	55	16	1	—
Bushbuck	31	12	1	—
Duiker	10	2	—	—
Waterbuck	13	6	—	—
Reedbuck	40	13	—	—
Mountain Reedbuck	18	4	—	—
Kudu	3	—	1	—
Warthog	26	—	—	—
Wildcat	1	—	—	—
Steenbuck	1	1	—	—
Otter	1	—	—	—
Inyala	1	—	—	—
Crocodile	1	—	—	—
Blue Wildebeest	35	3	—	—

Particulars concerning the species of animals, total number examined from Empangeni, the number showing either small piroplasma, trypanosoma, or microfilaria are given in the attached Table VII (*a*).

TABLE VII (*a*).

Game Animals.	Total Number Examined.	Number found infected with Small Piroplasms.	Number found infected with Trypanosama.	Number found infected with Microfilaria.
Zebra	50	18	—	—
Bushbuck	9	5	1	—
Duiker	12	6	—	—
Waterbuck	2	1	—	—
Reedbuck	3	0	—	—
Mountain Reedbuck	—	—	—	—
Kudu	1	—	—	—
Warthog	16	—	—	—
Antbear	1	1	—	—
Klipspringer	1	—	—	—
Blue Wildebeest	1	—	—	—

Details regarding the species of animal, total number examined from Umfolozi River; the number showing either small piroplasma, trypanosoma or microfilaria are given in the following Table VII (*b*).

TABLE VII (*b*).

Game Animal.	Total Number Examined.	Number found infected with Small Piroplasms.	Number found infected with Trypanosoma.	Number found infected with Microfilaria.
Zebra	22	7	—	—
Bushbuck	20	11	1	1
Duiker	28	19	—	—
Waterbuck	8	4	—	—
Reedbuck	6	2	—	—
Mountain Reedbuck	—	—	—	—
Kudu	4	3	—	—
Warthog	14	—	—	5
Steenbuck	1	—	—	—
Blue Wildebeest	4	2	—	—

In conclusion an appended list giving common and zoological names for the game animals dealt with is shown in Table VIII.

TABLE VIII.

Common Name.	*Zoological Name.*
Zebra	*Hippotigris quagga wahlbergi* Pocock.
Bushbuck	*Tragelaphus scriptus sylvaticus* Sparr.
Inyala	*Nyala Angasi* (Ang.).
Kudu	*Strepsiceros strepsiceros strepsiceros* Pall.
Waterbuck	*Cobus ellipsiprymnus* Ogilby.
Reedbuck	*Redunca arundinum arundinum* Bodd.
Mountain Reedbuck	*Redunca fulvorufula* (Afz.).
Duiker	*Sylvicapra grimmi grimmi* L.
Red Duiker	*Cephalophus natalensis natalensis* Smith.
Blue Wildebeest	*Gorgon taurinus taurinus* (Burch.).
Steenbuck	*Rhaphiceros campestris* Thunb.
Klipspringer	*Oreotragus oreotragus transvaalensis* Robts.
Warthog	*Phacochoerus aethiopicus sandevalli* Lönnb.
Antbear	*Orycteropus afer afer* (Pall.).
Wildcat	*Felis ocreata caffra* Desm.

Discussion. (See Table V.)

1. *Zebra.*—A small piroplasm had been described by Ross P.H. (1907) in Uganda and by Theiler (1909) in 1907 in the South African Zebra. Kudicke observed similar parasites according to Ollwig and Manteufel (1912). In a later publication Ross P.H. (1911) again mentions these parasites. Theiler considered them to be *N. equi*. The parasites seen by the writer resemble *N. equi* very closely and in one smear (see Fig. V1) ovoid forms showing division into four, producing the cross arrangement were seen. It this case it appears that the padasite dealt with is *N. equi*.

2. Bushbuck.—Small piroplasms resembling *Th. mutans* were found by Ross P.H. (1911) in a bushbuck from East Africa. In the examined material parasites of the same type were found in twenty-eight animals. In twenty-seven cases small piroplasms only were found and in one case small piroplasms together with Koch's bodies were seen. Do these parasites behave like *Th. mutans*? In other words do Koch's bodies develop in certain circumstances as a result of some unknown influence on the parasite or on the host? Or are the parasites seen in the one case of the *Th. parva* type?

The parasites seen in the abovementioned twenty-seven occasions resembled *Th. mutans* very closely. The length varied from 1.0μ to 2.0μ by 0.5μ to 1.0μ. The various forms observed were pearshaped, ringed, ovoid or commashaped, and the nucleus was situated at the wider extremity. Either one, two, three or four parasites were seen in a single cell. The actual process of multiplication is unknown but it would appear that multiplication into either two or four took place. The small piroplasms seen in abovementioned bushbuck varied in length from 0.5μ to 2.0μ by 0.2μ to 1.5μ. Various forms were found, namely pear, comma, ring and ovoid forms. The nucleus was distinct and situated at the wider extremity. The number of parasites seen in a single erythrocyte varied from one, two, three or four, and in one case as many as eight small piroplasms were found in a single cell. Division probably took place into either two or four. Whether the eight parasites found in the single cell originated from one or two parasites can not be stated definitely. The small piroplasms were seen in the blood, spleen and gland smears but Koch's bodies could only be demonstrated in the spleen smears. Both agamonts and gamonts were found but of the latter only a very few were seen. The Koch's bodies measured from 3.25μ to 10.0μ in

diameter. The majority of these plasma bodies were extracellular, but a few were intracellular. In the circumstances it is proposed to name this parasite *Theileria tragelaphi*. (See Figures IX, X, XI.)

3. *Duiker.*—Bettencourt and Borges (1909) described small piroplasms in the blood from a duiker in Africa. Bruce (1915) saw similar parasites in Nyassaland. Parasites of the *Th. mutans* type were observed by the writer in blood, spleen and gland smears, in some cases rare and in others fairly frequent. No plasma bodies were seen, not even in those spleen smears in which the parasites were fairly frequent. The parasites measure 1.0μ to 1.75μ by 0.5μ to 1.0μ. The forms seen were ringed, pearshaped, comma and ovoid. Not more than two parasites were ever seen in a single cell.

4. *Reedbuck.*—In the blood, spleen and gland smears small piroplasms, in some cases rare and in others fairly frequent, were seen in the Zululand material. These parasites measured 1.0μ to 1.8μ by 0.5μ to 0.8μ. Ovoid, comma and pearshaped forms were observed. The mode of multiplication is not known. Only one parasite was seen in a single cell.

5. *Mountain reedbuck, or Red Rhebuck.*—Similar parasites as described in the reedbuck were seen by the writer in the blood, spleen and gland smears.

6. *Blue Wildebeest.*—In the blood, spleen, and gland smears small pearshaped piroplasms were observed in rare numbers. The parasites varied in size from 1.5μ to 1.75μ by 0.5μ to 1.0μ. Not more than one parasite was seen in a single red cell. The mode in which this parasite multiplies was not seen. The occurrence of this parasite has not been mentioned before.

7. *Steenbuck.*—Small piroplasms of the *Th. mutans* type were found in rare numbers in the blood and spleen smears in one steenbuck. The parasite varied in length from 1.0μ to 1.75μ by 0.5μ to 0.8μ. Ovoid, pear and commashaped forms were seen. The nucleus was situated at the wider extremity, and only one parasite was found in a single erythrocyte. The manner in which the parasite multiplied is unknown. The presence of this parasite has not been referred to previously.

8. *Kudu.*—In the blood, spleen and gland smears parasites resembling *Th. mutans* were found in rare numbers. The organisms measured 1.0μ to 1.75μ by 0.5μ to 0.8μ. Only single parasites were seen in the red blood cells. The mode of multiplication is unknown. Only ovoid forms were seen.

9. *Antbear.*—Parasites resembling *Th. mutans* type were found in rare numbers in the blood and spleen smears. The parasites vary in size from 1.0μ to 2.0μ by 0.5μ to 0.6μ. Various forms, namely ovoid, ring and commashaped forms were observed. Not more than one parasite was found in a single erythrocyte. The method of multiplication is unknown. The nucleus is situated at the wider extremity. No mention of this parasite is made in the literature.

10. *Waterbuck.*—Rodhain (1916) describes small piroplasms resembling *Th. mutans* in a waterbuck (*Cobus defassa*) from East Africa. Schwetz and Collard (1929) describe similar parasites in the

blood from a waterbuck (*Cobus ellipsiprymnus*) from the Belgian Congo. Similar parasites were found in blood, spleen and gland smears from waterbuck from the three areas under discussion. The parasites varied in size from 1.0μ to 1.5μ by 0.5μ to 0.6μ. The probability that the parasite seen in *C. defassa* and *C. ellipsiprymnus* are one and the same parasite is very great. Ovoid, commashaped and pearshaped forms were seen and not more than one parasite was seen in a single erythrocyte. The mode of multiplication is not known.

11. *Warthog.*—Only microfilaria measuring about 0.11 mm. were found in blood, spleen and gland smears. A characteristic feature is the constant polychromasia in the smears. In a few cases Jolly bodies were also observed.

Conclusions.

1. *T. congolense* was found in three game animals, viz:—one zebra, one kudu and in one bushbuck. *T. vivax* was found in one bushbuck.

2. Small piroplasms were demonstrated in the zebra, bushbuck, duiker, waterbuck, kudu, blue wildebeest, reedbuck, mountain reedbuck, steenbuck and antbear. In the last six these parasites are recorded for the first time.

3. Microfilaria were found in one bushbuck and five warthogs.

As soon as circumstances permit it is proposed to study these parasites further and if necessary to name them.

LITERATURE.

Full lists of references to *Babesidae*, *Theileridae* and *Trypanosoma* of wild animals are to be found in the publications of Knuth and Du Toit (1921) and Wenyon (1926).

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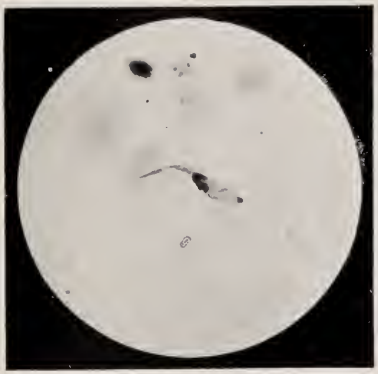


Fig I.—Kudu 68. *T. congolense* found in blood smear. Magnification 1,000 ×.

Fig. II.—Bushbuck 428. *T. vivax* found in blood smear. Magnification 1,100 ×.

Fig. III.—Zebra 197. *T. congolense* found in blood smears. Magnification 1,100 ×.

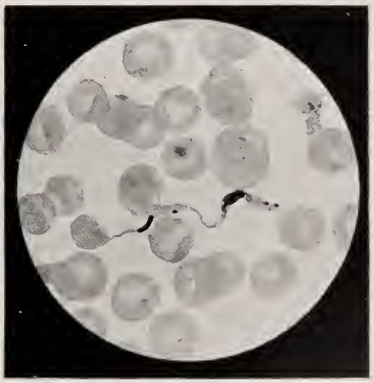


Fig. IV.—Zebra 197. *T. congolense* found in blood smear. Magnification 1,100 ×.

Fig. V.—Zebra 29. Small piroplasm found in blood smear. Magnification 1,000 ×.

Fig. VI.—Zebra 65. Four small piroplasms in a single erythrocyte found in blood smear. Magnification 1,000 ×.

Fig. VII.—Reedbuck 328. Small piroplasm found in blood smear. Magnification 1,100 ×.

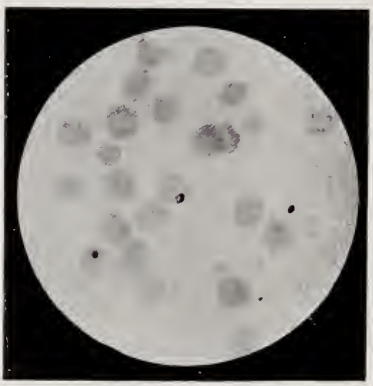


Fig. VIII.—Mountain Reedbuck 312. Small piroplasms found in blood smear. Magnification 1,100 ×.

Fig. IX.—Bushbuck 219. Koch's bodies (agamont, extracellular) found in spleen smear. Magnification 1,100 ×.

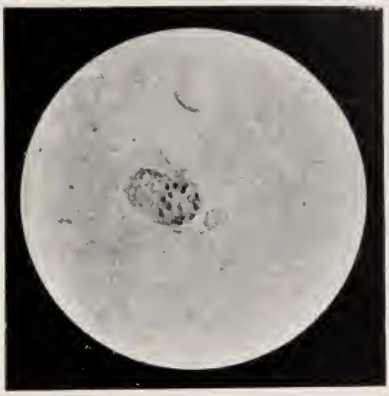


Fig. X.—Bushbuck 219. Koch's body (agamont, intracellular) found in spleen smear. Magnification 1,100 ×.

Fig. XI.—Bushbuck 219. Eight small piroplasms in a single erythrocyte found in spleen smear. Magnification 1,100 ×.

Fig. XII.—Bushbuck 26. Small piroplasm found in blood smear. Magnification 1,000 ×.

Fig. XIII.—Kudu 332. Small piroplasm found in blood smear. Magnification 1,100 ×.

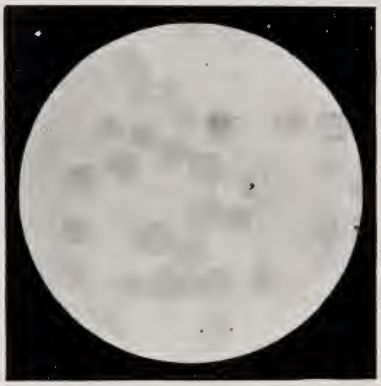


Fig. XIV.—Waterbuck 56. Small piroplasm in blood smear. Magnification 1,100 ×.

Fig. XV.—Bovine 2611. Th. mutans in blood smear. Magnification 1,100 ×.

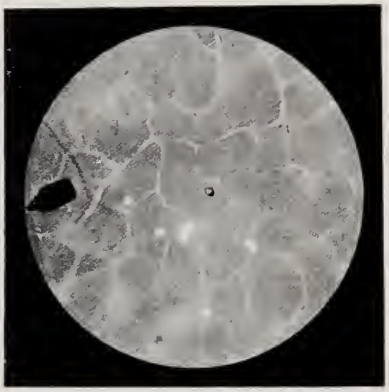


Fig. XVI.—Antbear 259. Small piroplasm in blood smear. Magnification 1,100 ×.

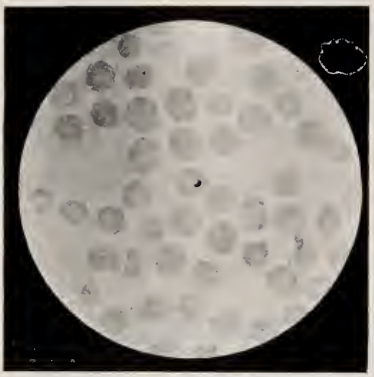


Fig. XVII.—Blue Wildebeest 358. Small piroplasm in blood smear. Magnification 1,100 ×.

Fig. XVIII.—Blue Wildebeest 174. Small piroplasm in blood smear. Magnification 1,100 ×.

Fig. XIX.—Duiker 365. Small piroplasms in spleen smear. Magnification 1,100 ×.

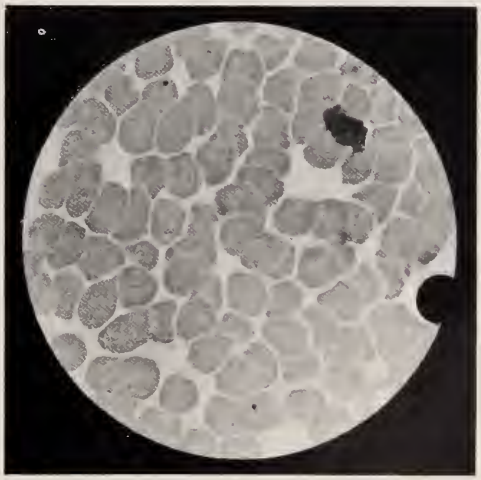


Fig. XX.—Steenbuck 296. Small piroplasms in blood smear. Magnification 1,100 ×.

Fig. XXI.—Warthog 418. Microfilaria in blood smear. Magnification 280 ×.

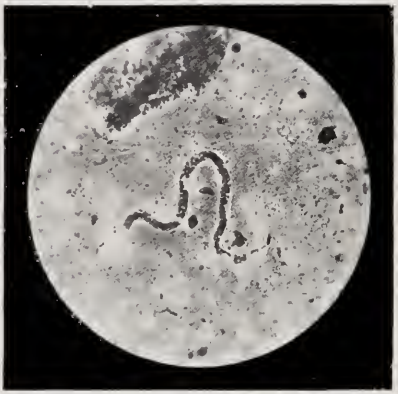


Fig. XXII.—Bushbuck 362. Microfilaria in blood smear. Magnification 280 ×.

Antimony Therapy in *Trypanosoma brucei* Infection of Horses.

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In a previous article by the author (1930) there were recorded the results obtained by the treatment with Antimosan of horses infected with *Trypanosoma brucei*.

This short article records further treatment of the disease with the same drug, the object being to determine more exactly the probable requirements as far as the size of the dose and the intervals between administration are concerned. The number of horses used for this purpose is small, but the information obtained by varying the doses and the intervals justifies the publication of the results.

Arrangement of the Experiment.

In all five horses were introduced into the experiment. The intervals of administration were one week, two weeks and three weeks. The doses were 3.75 gm. and 5.25 gm., repeated a number of times, and ascending doses of 3 gm., 6 gm., and 9 gm. Control of the results of the treatment was based on the result of the examination of stained blood smears, on the appearance of the temperature curve and on the Complement Fixation Test. In a few cases it was possible to test the sterility further by subinoculation into horses and reinoculation of the presumed sterile.

The table is arranged to give the various details in connection with the treatment of each horse.

Table I.

Antimosan Therapy.

Equines.	Weight in Kg.	No. of Doses and Dosage.	Intervals.	Total Used.	Result.
18095	352	6 doses of 5·25 gm.	3 weeks	31·5 gm.	Sterilization.
17989	431	6 ,, 3·75 gm.	3 ,,	22·5 gm.	Failure.
17990	423	8 ,, 3·75 gm.	2 ,,	30·0 gm.	,,
19431	374	8 ,, 5·25 gm.	2 ,,	42·0 gm.	Sterilization.
19079	308	3 ,, 3, 6, and 9 gm.	1 week	18·0 gm.	,,

Discussion.

The weights of the horse were obtained after the completion of the experiment, consequently the weights of the two failures were probably slightly higher, comparatively, than is indicated by the table, for, as a result of a successful treatment, there occurred an improvement in condition. However, even though the horses 17989 and 17990 were failures as far as sterilization was concerned, yet they also showed a definite improvement in condition. Thus, notwithstanding the slight disparity of weights, the results are regarded as comparable.

It can thus be seen that the dose of 3.75 gm., even though given repeatedly and at intervals of two and three weeks, would appear to be too small to produce sterilization in the two horses 17989 and 17990. In these two cases failure was determined by the detection of *T. brucei* in blood smears in the 9th week and 13th week respectively, by a positive Complement Fixation Test in both, and by the reappearance of the *T. brucei* temperature curves in the 11th week in both cases. Blood of horse 17989 was furthermore subinoculated into a number of other horses, it being used as a reservoir of *T. brucei*, always with positive results. This horse is at present, 10 months after cessation of treatment, in good condition, although occasional irregularities of temperature are noted.

Of the three horses that were sterilized, one was treated at one week intervals, one at two weeks and one at three weeks. Longer intervals were not feasible, because on account of the severity of *T. brucei* infection in horses, the reapperance of trypanosimes in the blood is associated with a rapid loss of condition and the development of marked symptoms. Trypanosomes were not found in these three horses during the intervals between treatment, but were found during the intervals in the two failures in one case as early as the 11th day. This finding might be regarded as additional evidence that the dose used in the failures was on the small side.

A difficulty in the study of the chemotherapy of trypanosomiasis is that of determining when an animal is sterilized. For example, in the case of horse 19431 and also of horse 18095 it is quite likely that sterilization had been obtained long before the cessation of treatment. There is some evidence that this was actually the case. This will be referred to again during this discussion. Of great interest in the matter of the dosage is the sterilization obtained in horse 19079. From the information obtained from the two failures, the first dose used, namely, 3 gm., may be regarded as too small to produce sterilization. Consequently sterilization must have been produced by either the 6 gm. or the 9 gm. dose. There could not, therefore, have been any interference with the efficacy of the subsequent doses by the administration of the first dose. The same probably holds good in the cases of the other two sterilized animals, for, in the previous article quoted above, the use of two doses each of 6 gm. at an interval of a week did not produce sterilization.

The ascending doses which totalled 18 gm. were the most economical in this experiment, but it is quite likely that a reduction in the total amount used in the other two cases of sterilization might be brought about by decreasing the number of doses. From the point of

handling necessary, which is always of importance in dealing with horses which are not as a routine handled daily, the use of large doses is indicated, whether of the same size or ascending cannot now be stated.

The most important and, as has already been pointed out, difficult point in the efficient control of the results obtained in the treatment of trypanosomiases is the determination of sterility. After treatment with Antimosan trypanosomes such as *T. congolense* and *T. brucei* become extremely rare in the blood. This is particularly the case in *T. brucei* infection of horses. Consequently negative blood-smear examinations is not of any great significance unless it be carried on over a long period and receives the support of other evidence, e.g. the temperature curve. But better still is the subinoculation of blood into horses which are then examined. The Complement Fixation Test and re-infection have also been utilized for the purpose. The former gives good results but, unfortunately, a horse does not become negative for some little time after sterilization. The latter was used in all three cases. It naturally results in the re-infection of the animal if it is sterilized, a condition which might not always be desirable even under laboratory conditions and never can be under field conditions. It is proposed to deal with each of these methods of determination of sterility in the three sterilized horses in turn.

Blood-smear examination showed that each of the three horses was positive before treatment was commenced. Smear examinations were carried out three times weekly. To illustrate the difficulty of detecting *T. brucei* which one meets with at times, one might mention horse 19079 which only showed *T. brucei* once in blood-smears over a period of 13 weeks. During the treatment of horse 19079 no trypanosomes were found. Complete smear examination subsequent to treatment was not carried out, for there were horses available for subinoculation. In horse 19431 smear examination was carried out during and for 12 weeks after cessation of treatment with negative results. In horse 18095 similarly for a period of 11 weeks negative results were obtained.

The temperature curve was available for horse 19079 for six weeks when re-infection was carried out, and for horses 19431 and 18095 for 17 weeks. The indications were that none of the three were infected. The temperatures were somewhat irregular in horses 19431 and 18095 during the last interval, i.e. before the administration of the last dose.

The Complement Fixation Test* was carried out on the horses in the experiment. They all gave positive results either before or during treatment. The ultimate test, of course, in horses 17989 and 17990 was positive. In horse 19079 the test was positive two days before the last dose of Antimosan, but was negative on the 28th day after cessation of treatment. This means that a negative result was obtained on the 42nd day after commencement of the treatment. In horse 19431 the test was positive after four doses had been given and became negative 11 weeks after the last dose, being doubtful during the 7th week. In horse 18095 the test was negative after the third

* I wish here to thank Dr. Robinson of this Institution for carrying out this test for me.

dose and also during the 6th week after the last dose. The former of the two results in horse 18095 is rather surprising, for it seems to indicate that sterilization must have been produced by either the first or the second dose, a possibility which is not supported by the temperature curve. At any rate, it can be said that the Complement Fixation Test becomes negative within a short period after sterilization.

As further cases of *T. brucei* infection were required for another experiment the opportunity was taken to test the sterility of horse 19079 by the injection of 50 c.c. of blood of this horse into horses 18095 and 19431. These two were then kept under observation for 15 days with negative results as far as the blood-smears and temperatures were concerned. Their susceptibility was proved by the injection of 50 c.c. of blood of horse 17989, one of the failures. In both cases trypanosomes were found in the blood-smears on the 4th day, temperatures of 106 and 106.2 respectively being recorded on that day.

The observations in connection with the susceptibility of horses 18095 and 19431 and subsequently of horse 19079 which when injected with blood of horse 17989 also became infected, are regarded as a good method of determining sterility, because the same strain of *T. brucei* was utilized.

A further important and interesting point in connection with the sterilized horses is their lack of resistance to re-infection. The three examples quoted in the previous paragraph show clearly that sterilized horses can be easily re-infected. The difficulty again arises in connection with any calculation of the period of time that elapsed, in these three cases, from time of sterilization to time of re-infection. The following table, however, gives the range of the period:—

TABLE II.

Equines.	Treatment Commenced.	Treatment Stopped.	Re-infection.	No. of Days Sterilization to Re-infection.
19079.....	11/7/30	25/7/30	5/9/30	42 to 56
18095.....	10/1/30	19/4/30	5/9/30	143 to 242
19431.....	10/1/30	25/4/30	5/9/30	137 to 242

The case of horse 19079 is particularly interesting. The smaller figure, 42 days, is more likely to be the correct one. Thus after sterilization this horse was susceptible to reinfection to the same strain of *T. brucei* in apparently six weeks. For practical purposes such a period of immunity, even if it were present, would be of no value. It should be noted in conjunction with this, that the Complement Fixation Test gave a negative result on the 42nd day after commencement of treatment and on the 28th day after cessation. From this it would appear that the 42 days period could be somewat decreased. One comes then to the conclusion that there is probably no absolute immunity in connection with recoveries from *T. brucei* infection of

horses. The same conclusion was arrived at in connection with *T. congolese* infection of bovines. And, furthermore, it may definitely be stated that there is produced little or no resistance to infection by the use of Antimosan.

Summary.

1. Of five horses treated with varying doses of Antimosan at different intervals three were sterilized.

2. The methods of determining sterility are discussed, as is also immunity in *T. brucei* infection of horses.

REFERENCES.

PARKIN, B. S. (1930). Antimony Therapy in Equine Trypanosomiasis (*T. brucei*). *16th Rept. of the Dir. of Vet. Ser. and An. Industry*, pp. 55-59.

Antimosan Therapy in *Trypanosoma congolense* Infection of Sheep.

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In articles by the author in this Report and in the 16th Report of the Director of Veterinary Services and Animal Industry, the use of Antimosan in the treatment of *Trypanosoma congolense* and *Trypanosoma vivax* infection of bovines and of *Trypanosoma brucei* infection of equines is described. The treatment of ovine trypanosomiasis caused by *Trypanosoma congolense* is dealt with in this article.

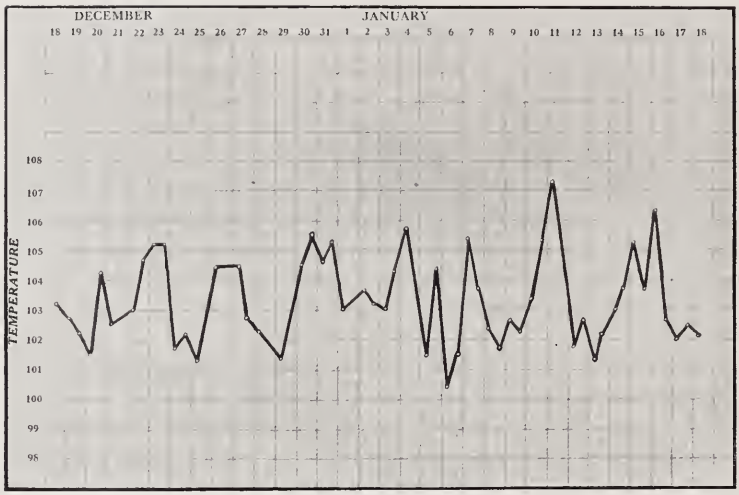
No difficulty was experienced in the production of the infection in sheep by the intravenous injection of the blood of bovines or sheep infected with *T. congolense*. The sheep before treatment was instituted were kept for varying times and consequently the results obtained with the same doses are probably not strictly comparable, for it has been observed in bovines that those which had been infected for varying periods before treatment was instituted gave different responses to treatment.

A number of the infected sheep died before treatment was commenced, but the mortality was undoubtedly influenced, in some cases, by concurrent heartwater. In general the infection produced in the sheep was of a subacute type, but, in many cases, the ultimate condition was of a chronic nature characterised by a loss of condition, an anaemia and a temperature curve showing fairly regular marked remissions and exacerbations. (See chart I.)

For the experiments sixty-three sheep were utilized. This number does not include those sheep which died before treatment was instituted, but does include two sheep (S.25499 and S.28935), which died two and three days respectively after the injection of doses of Antimosan. Death in both cases was due to trypanosomiasis, the institution of treatment having been delayed too long.

CHART 1.

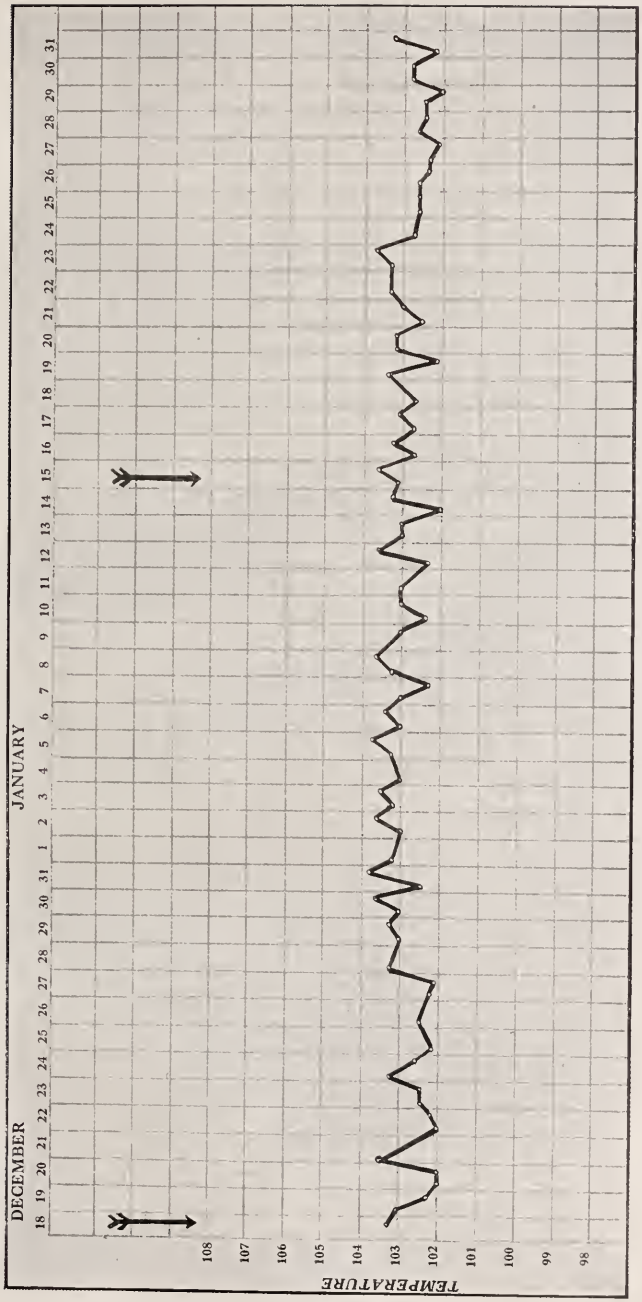
Sheep 20949. *T. congolense.*



Temperature curve of untreated case

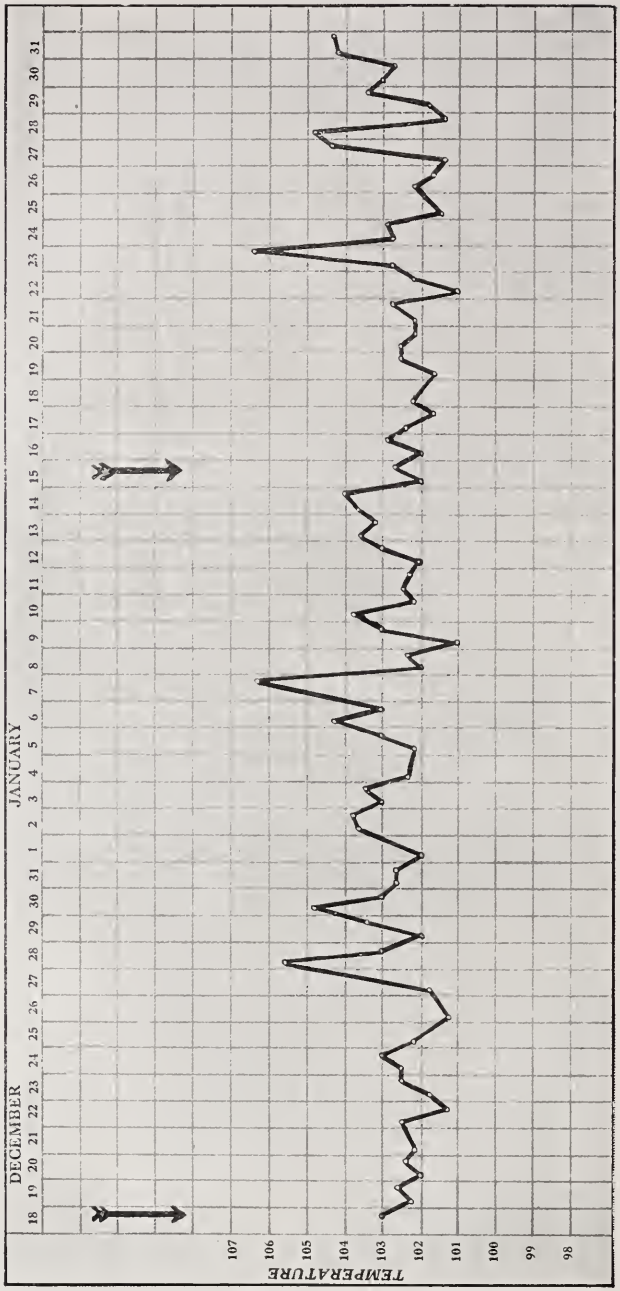
CHART II.

Sheep 2868. *T. congolense*.



Sterilization with two injections of 7 c.cm. of antimosan

CHART III.
Sheep 26759. *T. congolense*.



Failure with two injections of 7 c.cm. of antimosan.

The Antimosan used in all the experiments was the sodium salt in 12 per cent. solution. The doses in all the tables are in cubic centimetres of this solution. The Antimosan was administered, with a hypodermic syringe and needle, subcutaneously, at the medial aspect of the right thigh. Notwithstanding that no disinfection or washing of the site was carried out at the time of the injection of the drug, yet in only one case did an abscess, which was small, result. The same site was always used, even when a series of weekly injections was given.

The majority of the sheep weighed approximately 35 Kg., the lightest being about 30 Kg. and the heaviest about 42 Kg.

The results of the treatment, i.e. whether sterilization had been obtained or not, were determined from the examination of the temperature curve, temperatures being taken twice daily, and of stained blood smears prepared three times weekly. When both the temperature curve and the blood smears did not indicate clearly that the sheep were free from infection, blood smears were continued for a period which in some cases extend to more than two months after cessation of treatment. It is considered that this method of determination of sterility gives reliable results, although it would have removed all doubt if sub-inoculations from the sheep had been carried out. Charts II and III illustrate successful and unsuccessful treatments of two sheep.

In the tables the sheep are grouped according to similarity of doses of Antimosan. In some cases, when sheep were given doses which later were increased, the recording of the results may be included in more than one table, first in the tables recording the smaller doses and then in the tables of the larger doses. This procedure, however, is only carried out in those cases where it was possible to determine, before the administration of the larger doses, the failure of the smaller doses and where the interval between the smaller doses and the larger ones was at least six weeks.

The percentage of sterilization for the various groups is recorded in the last table.

EXPERIMENT 1.

TABLE I.

D.O.B. No.	Dosage in c.c.				Interval in days.	Blood Smear.	Result.
25499...	3	—	—	—	—	—	Died on second day.
26125...	3	5	5	5	7	Positive.	Unsuccessful.
27471...	3	5	5	5	7	Positive.	Unsuccessful.

Discussion.

The blood smears obtained during the periods between the injections were negative, but trypanosomes were detected in the smears on the 34th and 11th days respectively after the last injections. The increase in dosage from 3 to 5 c.c. was instituted on account of the very slight influence the 3 c.c. dose had on the temperature.

EXPERIMENT 2.

In this series the doses were similar to those of Experiment 1, but an additional dose of 5 c.c. was given.

TABLE II.

T. congolense. Antimosan Therapy.

D.O.B. Nos.	Dosage in c.c.					Intervals in days.	Blood Smear.	Result.
24784...	3	5	5	5	5	7	Positive.	Unsuccessful.
25649...	3	5	5	5	5	7	Negative	Sterilization.
26054...	3	5	5	5	5	7	Positive.	Unsuccessful.
28551...	3	5	5	5	5	7	—	Unsuccessful.
28807...	3	5	5	5	5	7	Negative	Sterilization.
28834...	3	5	5	5	5	7	Positive.	Unsuccessful.
28957...	3	5	5	5	5	7	Negative	Sterilization.

Discussion.

The introduction of an additional 5 c.c. dose resulted in the sterilization of three of the sheep. Probably further success would have been obtained if the treatment had been persisted in.

EXPERIMENT 3.

As some success had resulted from the employment of four 5 c.c. doses after one 3 c.c. dose, this series was given four 5 c.c. doses.

TABLE III.

T. congolense. Antimosan Therapy.

D.O.B. Nos.	Dosage in c.c.				Intervals in days.	Blood Smear.	Result.
25503...	5	5	5	5	7	Positive.	Unsuccessful.
26323...	5	5	5	5	7	Positive.	Unsuccessful.
26519...	5	5	5	5	7	Positive.	Unsuccessful.

Discussion.

If the result obtained from this experiment be compared with those of Experiment 2, it would appear that the administration of the preliminary 3 c.c. dose had some favourable influence.

EXPERIMENT 4.

This is a repitition of the previous experiment with, however, an additional 5 c.c. dose.

TABLE IV.

T. congolense. Antimosan Therapy.

D.O.B. Nos.	Dosage in c.c.					Intervals in days.	Blood Smear.	Result.
27660...	5	5	5	5	5	7	Negative	Sterilization.
28065...	5	5	5	5	5	7	Positive.	Unsuccessful.
28317...	5	5	5	5	5	7	Positive.	Unsuccessful.
28386...	5	5	5	5	5	7	Positive.	Unsuccessful.
28524...	5	5	5	5	5	7	Negative	Sterilization.
28996...	5	5	5	5	5	7	Positive.	Unsuccessful.
25539...	5	5	5	5	5	7	Positive.	Unsuccessful.
26629...	5	5	5	5	5	7	Positive.	Unsuccessful.
27547...	5	5	5	5	5	7	Positive.	Unsuccessful.

Discussion.

The results obtained are no better than Experiment 2. The continuation of the use of a 5 c.c. dose does not present much hope of success. If further doses of 5 c.c. were to be given, the treatment for practical purposes would be too drawn out and too expensive.

EXPERIMENT 5.

The next series was arranged to give an ascending dosage with a maximum of 10 c.c. This served also to determine the tolerance of the sheep to fairly large doses.

TABLE V.

T. congolense. Antimosan Therapy.

D.O.B. Nos.	Dosage in c.c.				Intervals in days.	Blood Smear.	Result.
25751...	3	5	10	10	7	—	Sterilization.
25770...	3	5	10	10	7	—	Sterilization.
19151...	5	5	10	10	7	—	Sterilization.
25502...	5	5	10	10	7	—	Sterilization.
27526...	5	5	10	10	7	—	Sterilization.

Discussion.

No ill effects were observed as the result of the use of 10 c.c. doses. Trypanosomes were detected in smears before the third dose in all the sheep, but not subsequently. Probably the good results are, in this experiment, not so much dependent on the presence of the two first doses as due to the use of 10 c.c. as end doses.

EXPERIMENT 6.

For this series a slight increase was made in the dosage to 7 c.c. Two sheep were given single doses, while the remainder received two doses at an interval of 28 days.

TABLE VI.

T. congolense. Antimosan Therapy.

D.O.B. No.	Dosage in c.c.		Intervals in days.	Blood Smear.	Result.
20949....	7	—	—	Positive...	Unsuccessful.
21065....	7	—	—	Negative...	Sterilization.
23487....	7	7	28	Positive...	Unsuccessful.
25734....	7	7	28	Negative...	Sterilization.
25793....	7	7	28	Negative...	Sterilization.
26759....	7	7	28	Positive...	Unsuccessful.
27438....	7	7	28	Positive...	Unsuccessful.
28275....	7	7	28	Negative...	Sterilization.
28658....	7	7	28	Negative...	Sterilization.
28799....	7	7	28	Negative...	Sterilization.
28871....	7	7	28	Negative...	Sterilization.
28881....	7	7	28	Negative...	Sterilization.
28919....	7	7	28	Negative...	Sterilization.
28979....	7	7	28	Negative...	Sterilization.

Discussion.

The increase of the dose to 7 c.c. and the interval to 28 days gave much better results. There was thus obtained increased efficiency with a decrease in the total c.c. of Antimosan used.

EXPERIMENT 7.

The next series was devised for the purpose of determining whether a single dose of 10 c.c. would give the required result. In this experiment, in addition, were included those cases which were failures in previous experiments.

TABLE VII.

T. congolense. Antimosan Therapy.

D.O.B. Nos.	Dose in c.c.	Failure from.	Blood Smear.	Result.
24694...	10	—	Negative.....	Sterilization.
28063...	10	—	Negative.....	Sterilization.
28641...	10	—	Negative.....	Sterilization.
28999...	10	—	Negative.....	Sterilization.
28834...	10	Experiment 2.....	Negative.....	Sterilization.
26054...	10	Experiment 2.....	Negative.....	Sterilization.
24784...	10	Experiment 2.....	Negative.....	Sterilization.
25539...	10	Experiment 4.....	Negative.....	Sterilization.
26323...	10	Experiment 3.....	Positive.....	Unsuccessful.
26629...	10	Experiment 4.....	Negative.....	Sterilization.
27547...	10	Experiment 4.....	Negative.....	Sterilization.
28386...	10	Experiment 4.....	Negative.....	Sterilization.
28996...	10	Experiment 4.....	Negative.....	Sterilization.
28317...	10	Experiment 4.....	Negative.....	Sterilization.
28551...	10	Experiment 2.....	Negative.....	Unsuccessful.
26519...	10	Experiment 3.....	Negative.....	Unsuccessful.
28935...	10	Experiment 3.....	Negative.....	Died on third day.

Discussion.

Rather disappointing was the failure of the three sheep which had failed in previous experiments. The four sheep which had not been subjected previously to treatment were all sterilized.

EXPERIMENT 8.

As fairly good results had been obtained by the long interval administration of 7 c.c., this series was arranged also at long intervals with a commencing small dose, a commencing large dose, and two large doses.

TABLE VIII.

D.O.B. Nos.	Dosage in c.c.		Intervals in days.	Failure From.	Blood Smear.	Result.
28551..	10	10	28	Experiment 7..	Positive.	Unsuccessful.
26519..	10	10	28	Experiment 7..	Negative	Sterilized.
23556..	5	10	28	—	Negative	Sterilized.
23821..	5	10	28	—	Positive.	Unsuccessful.
24114..	5	10	28	—	Negative	Sterilized.
25661..	5	10	28	—	Negative	Sterilized.
25808..	5	10	28	—	Negative	Sterilized.
28341..	5	10	28	—	Negative	Sterilized.
28497..	5	10	28	—	Negative	Sterilized.
28796..	5	10	28	—	Negative	Sterilized.
28943..	5	10	28	—	Negative	Sterilized.
28977..	5	10	28	—	Negative	Sterilized.
25739..	10	5	28	—	Negative	Sterilized.
27398..	10	5	28	—	Positive.	Unsuccessful.
27422..	10	5	28	—	Positive.	Unsuccessful.
28795..	10	5	28	—	Positive.	Unsuccessful.
28828..	10	5	28	—	Negative	Sterilized.
28923..	10	5	28	—	Negative	Sterilized.

Discussion.

The first two sheep were treated after failure to a series of small doses which had been given at weekly intervals and one 10 c.c. dose. In both cases there was an interval of 21 days before the two 10 c.c. doses were given. The 5 c.c.–10 c.c. doses at 28 days interval were very much more successful than the arrangement 10 c.c.–5 c.c.

SUMMARY.

Table IX is compiled for the purpose of illustrating the comparison of the various arrangement of doses, intervals at which they were given and the percentage sterilized.

TABLE IX.

T. congolense. Summary of Treatment.

Doses in c.c. of a 12% solution of Antimosan.					Interval in days.	Reference.	No. of Sheep.	No. Sterilized.	% Sterilized.
3	5	5	5	—	7	Experiment 1..	2	0	0
3	—	—	—	—	—	Experiment 1..	1	0	0
3	5	5	5	5	7	Experiment 2..	7	3	43
5	5	5	5	—	7	Experiment 3..	3	0	0
5	5	5	5	5	7	Experiment 4..	9	2	22
3	5	10	10	—	7	Experiment 5..	2	2	100
5	5	10	10	—	7	Experiment 5..	3	3	100
7	—	—	—	—	—	Experiment 6..	2	1	50
7	7	—	—	—	28	Experiment 6..	12	9	75
10	—	—	—	—	—	Experiment 7..	17	13	76
10	10	—	—	—	28	Experiment 8..	2	1	50
5	10	—	—	—	28	Experiment 8..	10	9	90
10	5	—	—	—	28	Experiment 8..	6	3	50

Two doses or two end doses of 10 c.c. of Antimosan whether given at 7 or 28 days interval, sterilized the sheep of trypanosomes in all cases except one. The percentage sterilized by the single dose of 10 c.c. was good, especially when it is taken into consideration that one of the failures was due to the too late institution of the treatment. The smaller doses gave disappointing and unsatisfactory results. The 7 c.c. dose would probably in practice be a suitable average one, but if sterilization were desired it would be necessary to give a third one. This dose was not tried out at the 7-day interval.

CONCLUSION.

1. For sterilization of sheep infected with *Trypanosoma congolense* the dose of the 12 per cent. solution of the sodium salt of Antimosan necessary would probably be 10 c.c., i.e. 1.2 gm., repeated once or twice at an interval of 28 days.

2. A similar dose would probably produce sterilization if given at 7-day intervals.

3. The 7 c.c. dose of Antimosan gave good results, whereas the 5 c.c. dose gave unsatisfactory results.

4. The practical and economical dosage for the production of sterilization is the 10 c.c. repeated.

5. The approximate dosage per kg. live weight corresponding to a 10 c.c. dose of the 12 per cent. Antimosan used is 0.03 gm. of Antimosan.

Styrylquinoline No. 314 in Trypanosomiases.

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

Published results by Browning, Cohen, Ellingworth, and Gulbransen (1929) on the trypanocidal properties of various derivatives of Anil and Styryl Quinoline have shown these to be capable of producing sterilization in rabbits and mice infected with *Trypanosoma brucei*. The derivatives which gave most satisfactory results were Nos. 8 and 90. The strains of *T. brucei* used were Prowazek's and *feror*.

The Director of this Institution arranged with Professor Browning that derivative No. 90, which was regarded as being slightly more suitable than No. 8, be further tested against *T. brucei* infection of horses and *T. congolense* infection of bovines. Accordingly a supply of compound No. 314 was forwarded for the purpose. This compound is 2 (p-acetylamino-styryl) -dimethylanimo-quinoline methosulphate. Compound No. 90 is the corresponding methochloride.

Large doses of No. 90 (0.02–0.3 gm. per Kilo of body weight) were effective but frequently produced toxic effects in the animal injected. Smaller doses (0.004–0.0075 gm. per Kilo of body weight) if repeated several times were efficacious and well tolerated. The recommendation thus was that fractional doses be given at weekly intervals until six or more doses had been administered.

In the rabbits and mice the drug was given subcutaneously in a watery solution. The intravenous method was not utilized on account of the possibility of the production of fatal results. The subcutaneous injections did not produce any observable irritation of the subcutaneous tissue. In the human subject doses of 0.003 gm. per kilo given intramuscularly have been well tolerated.

Compound No. 314, which was the one received for test, has a solubility of 3.5 per cent. vol. It was recommended that it be given in watery solution in doses similar to those used in No. 90.

T. brucei Infection of Horses.

Five horses were utilised for the purpose of testing the efficacy of No. 314 in *T. brucei* infection of horses. No control was kept, as the virulency of the strain of *T. brucei* used has been well established in previous chemotherapeutical tests by the writer (1930). Furthermore, the one horse which did not respond to the treatment died of trypanosomiasis. The strain of *T. brucei* used was obtained from a natural case of the disease in a donkey.

The dose of No. 314, namely, 0.004 gm. per Kg. of body weight, was the same, with one exception, for all the cases treated. The intervals between administration were, in every case, one week. But variations were made in the number of doses given and, in an endeavour to overcome the difficulty of irritation to the tissues, variations were also made in the strength of the solutions. No attempt was made to determine whether a dose smaller than that recommended could be efficaciously employed.

The table gives the details in connection with the experiment and the charts (I and II) illustrate two cases, one a recovery and one a failure.

TABLE 1.

T. brucei. Treatment with No. 314.

Equine.	Weight in Kg.	Infection Date.	Commencement of Treatment.	No. of Doses and Dosage.	Intervals.	Remarks.
18095	352	5/9/30	12/9/30	2 doses of 1·4 gm.	1 week	Sterilization.
19323	454	5/9/30	12/9/30	2 doses of 1·8 gm.	,,	Died 5/11/30, Tryp. seen.
18022	363	5/9/30	27/9/30	2 doses of 1·5 gm. and 1 dose of 0·8 gm.	,,	Sterilization.
19431	374	5/9/30	12/9/30	3 doses of 1·5 gm.	,,	,,
17990	423	9/12/29	15/8/30	6 doses of 1·6 gm.	,,	,,

Discussion.

Horse 17990, the first one treated, was given in all six doses. The second dose was given intravenously and produced alarming symptoms. Immediately after the injection the horse dropped to the ground with symptoms of marked respiratory distress, frequent pulse and muscular twitchings. It did not recover sufficiently to stand up until after an hour. This was the only case when the drug was administered by any other route than the subcutaneous. After the treatment had commenced, *T. brucei* was not found in blood smears, and the temperature from then remained normal except for a short period of three days, due most likely to dermatitis gangrenosa produced by the injection. It is quite likely that sterilization was obtained prior to the cessation of treatment, for the complement fixation test was already negative by the 13th day, being doubtful on the 6th day.

Of the two horses which were given two doses one only was sterilized. This one gave a negative complement fixation test, negative blood smear examination for 10 weeks and normal temperatures for same period. The other horse, 19323, was not sterilized, trypanosomes being found on the 13th day and subsequently. It is of interest to note that the latter horse had not previously been in a *T. brucei* experiment, whereas horse 18095 was one which had been

sterilized and re-infected just prior to its introduction into this experiment. One is forced, therefore, to take into consideration the possibility of the previous infection having some influence on the treatment with No. 314. The two remaining horses, 18022 and 19431, both gave negative complement fixation tests and negative smears examinations and normal temperatures for seven and nine weeks respectively. Horse 18022 received for its third dose approximately half the other doses. As this horse had not been in any previous chemotherapeutical tests, it is an example of sterilization with two and a half doses uninfluenced by any former treatment.

There is thus good evidence to claim for No. 314 the possibility of bringing about sterilization in *T. brucei* infection of horses in a dose of 0.004 gm. per Kg. of body weight if more than two doses be given and, in some cases, although other factors might be influencing the result, with two doses.

Consequently it is much to be regretted that such excellent results lose some of their value by the fact that invariably the subcutaneous injection produces severe, often alarming, destruction of tissues below the site of injection. In every case there occurred swellings, most of which became abscesses, often deep-seated. In some also there was a dermatitis which became gangrenous. An endeavour was made to overcome this disability by varying the concentration, the vehicle and the mode of administration. The drug thus was given in percentages varying from 1 to 3.4, with and without massage during injection and in saline, distilled water and 10 per cent. glucose. The injection of the solution in small quantities at a number of sites resulted in only slight swellings. Also when the drug was given subcutaneously in sterile olive oil the swellings were only minor.

The swellings consequent on the injection of the drug often took two or more days to become evident. Prior to this the site of injection had a higher local temperature and a fairly well delineated slightly raised area.

T. congolense Infection of Bovines.

Compound No. 90 had been tested out on mice infected with *T. congolense*. No therapeutic action was detected.

To test compound No. 314 two bovines were used, a third being kept as a control. The drug was given at the same rate as in the horses, namely 0.004 gm. per Kg. of body weight. The injections were carried out on the left side of the neck, the subcutaneous route being employed. The solution was a 1 per cent. one in distilled water.

The two bovines, 3637 and 3684, showed no apparent response to the injections. The temperature curves were not influenced, the temperatures remaining high, and *T. congolense* were in both cases found in smears made 24 hours after the administration. On the 3rd day they were recorded as frequent.

In both the injected animals swellings occurred commencing at the site of injection and passing backwards to the posterior abdominal region and across to the opposite side. These swellings persisted for some weeks but no sloughing took place.

The bovines were not given a second injection.

SUMMARY.

1. Styrylquinoline No. 314 is an efficient sterilizing drug for use in *T. brucei* infection of horses.

2. The dose is 0.004 gm. per Kg. of body weight and more than two doses at an interval of one week should be used.

3. The drug when given subcutaneous produces marked irritation of the tissues and when given intravenously toxic symptoms.

4. It does not appear to have any therapeutical action on *T. congolense* infection of bovines.

5. In bovines it produces marked irritation of the tissues when given subcutaneously.

LITERATURE.

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CHART I.

Horse 18022.—*T. brucei.*

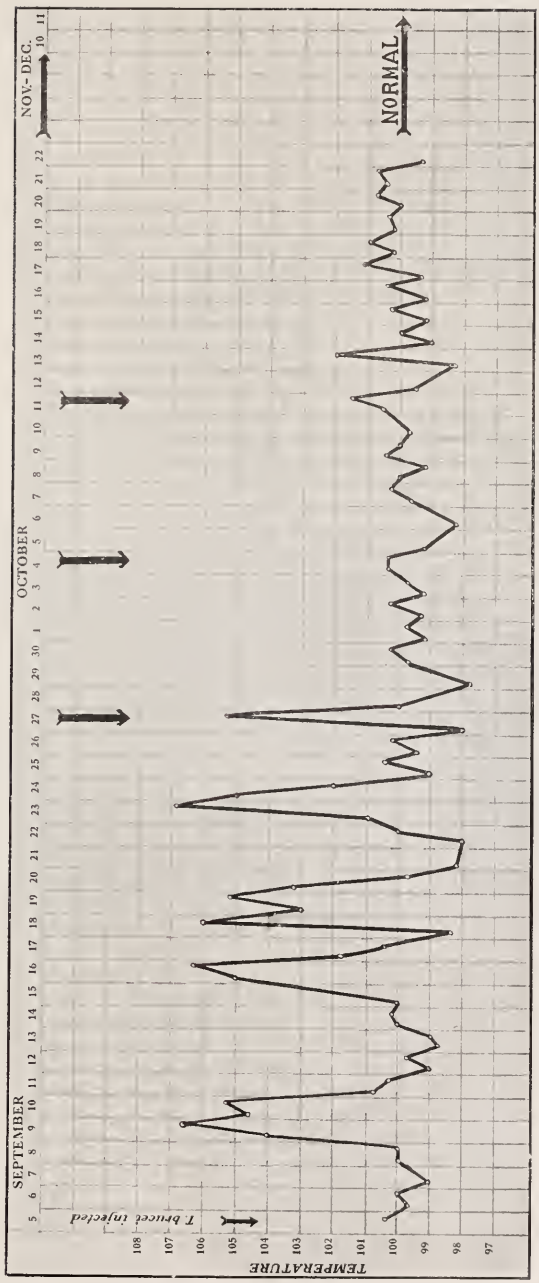
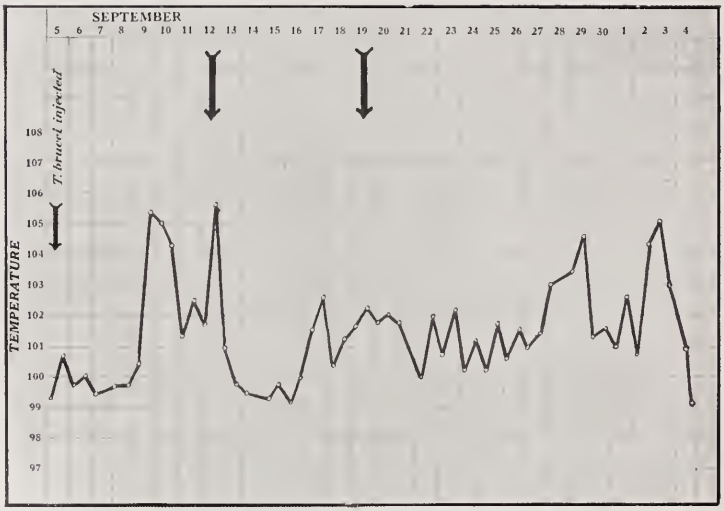


CHART II.

Horse 19323.—*T. brucei.*



A Note on the Diagnosis of Bovine Trypanosomiasis.

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

During the course of the experimental work on bovine trypanosomiasis caused by *Trypanosoma congolense* and *Trypanosoma vivax* described in last year's Report, the opportunity was taken to determine, under conditions of artificial infection, the relative frequency of the occurrence of these trypanosomes in blood and gland smears and also the period of incubation judged by appearance of the trypanosome in the smears.

Period of Incubation.

T. congolense infection was produced in the majority of the bovines by the inoculation, subcutaneously, of infected citrated guinea pig blood. A few were infected by the subcutaneous injection of blood obtained from an infected bovine. In the case of *T. vivax* infection transmission was effected by the injection subcutaneously of the blood of an infected bovine. Not a single failure in the transmission of either of these infections was encountered. To determine the earliest appearance of trypanosomes, stained blood and gland smears prepared daily were submitted to a careful examination. Table I gives the details in connection with these examinations. Figures of gland smear examination in *T. congolense* infection are available only for five bovines.

TABLE I.

T. congolense and *T. vivax*.—Period of Incubation.

Bovine.	Species of Trypansome.	Source of of Trypanosome.	Period of Incubation determined from:	
			Blood Smear.	Gland Smear.
2634	T. *congolense*.....	Guinea-pig.....	7	11
2639	,, ...	,, ...	6	9
2702	,, ...	,, ...	6	15
2709	,, ...	,, ...	7	11
2714	,, ...	,, ...	7	9
2464	,, ...	,, ...	6	—
2468	,, ...	,, ...	7	—
2473	,, ...	,, ...	5	—
2471	,, ...	,, ...	6	—
3506	,, ...	,, ...	7	—
3525	,, ...	,, ...	6	—
3527	,, ...	,, ...	5	—
3542	,, ...	,, ...	4	—
3627	,, ...	,, ...	4	—
2715	*T. vivax*.....	Bovine.....	14	8
2727	,, ...	,, ...	14	7
2743	,, ...	,, ...	16	6
2765	,, ...	,, ...	14	8
2766	,, ...	,, ...	15	11

Discussion.

In some of the above cases a shorter incubation period than that represented by the figures might have been obtained if smears had been made on Sundays. In the case of the first five *T. congolense* bovines and all the *T. vivax* bovines smears were made on the 4th, 6th and subsequent days but not on the 5th day after inoculation. The few bovines which were infected with *T. congolense* by the injection of blood from an infected bovine showed long incubation periods, the shortest determined being 14 days.

Comparison of Blood and Gland Smears.

The relative frequency of the occurrence of trypanosomes in blood and gland smears in infected bovines before the institution of treatment is summarized in Table 2 and 3. In every case under examination actual counts were made of the number of trypanosomes present in 50 fields of blood smears and 25 fields of gland smears. It is realized that the figures do not represent a high degree of accuracy on account of the varying thickness of the smears from day to day, but as the counts were made over a large number of fields the ultimate results do give a fair indication of the relative frequency of the two species in gland and blood smears. The smears were all made by the same assistant. A cypher represents a negative examination after a prolonged search.

TABLE II.

T. congolense.—Smear Examination.

Bovine.	Blood Smears.				Gland Smears.				Highest Count in:	
	No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	50 Blood Fields.	25 Gland Fields.
2634.....	900	1,730	18	0	450	31	6	12	414	11
2639.....	350	779	7	0	175	14	3	4	216	9
2702.....	850	558	17	0	425	8	7	10	78	2
2703.....	1,050	1,326	21	0	525	17	9	12	221	7
2714.....	3,750	3,241	68	7	1,500	38	22	38	542	8
TOTAL.....	6,900	7,634	131	7	3,075	108	47	76	542	11

Discussion.

The above figures demonstrate clearly that the diagnosis of *T. congolense* infection should be based on blood smear and not gland smear examination, for in the case of blood smear examination one trypanosome is found on an average in slightly less than one field whereas in case of gland smears 1 trypanosome is found only after examination of approximately 28 fields. Furthermore, the proportion of positive smears to negative smears in the examination of blood smears is approximately 19 to 1, whereas in examination of gland smears it is about 1 to 1.6. Several high counts were obtained in examination of some blood smears, but in case of the gland smears, the counts were always low.

TABLE III.

T. vivax.—Smear Examination.

Bovine.	Blood Smears.				Gland Smears.				Highest Count in:	
	No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	50 Blood Fields.	25 Gland Fields.
2715.....	500	5	5	5	250	50	7	3	1	19
2727.....	1,050	12	3	18	500	139	16	4	6	39
2743.....	800	1	1	15	375	111	10	5	1	77
2765.....	400	4	3	5	200	552	7	1	2	436
2766.....	800	12	5	13	450	155	13	5	8	46
TOTAL.....	3,650	34	17	56	1,775	1,007	53	18	8	436

Du Toit (1929) recommends the examination of stained gland smears as the method of diagnosing *T. vivax* infection in cattle. The figures of the last table support his results in a striking manner. In the blood smear series it is seen that it is necessary to examine

approximately 107 fields to detect one trypanosome, whereas the examination of not quite two fields, in the case of gland smears, is necessary for this purpose. Also there were, in the blood smear series, 56 negative smear examinations in every 73 examined, while in the case of the gland smear examination only 18 were negative of a total of 71. A remarkably high count of 436 trypanosomes in 25 fields was obtained in a gland smear. The highest obtained in a blood smear was only 8 trypanosomes in 50 fields.

When a relapse occurred after treatment in *T. congolense* infection, trypanosomes were always more numerous in the blood smears than in the gland smears. Table IV gives the details in connection with the examination of bovine 2634 in which animal treatment did not bring about sterilization.

TABLE IV.

Bovine 2634, *T. congolense*.—Smear Examination During and After Treatment.

Blood Smear.				Gland Smear.				Highest Count in:	
No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	No. of Fields.	No. of Tryps.	No. of + Smears.	No. of − Smears.	50 Blood Fields.	25 Gland Fields.
1,300	4,858	26	0	625	32	8	17	2,425*	12

* This figure was calculated from a count over 20 fields.

In this bovine, the only one in which a count was continued for any length of time, the disparity previously noted between blood and gland smear examination is still more marked. Nearly four trypanosomes were found per field of the blood smears, whereas it was necessary to examine on an average nearly 20 fields to find one trypanosome in the gland smears. The highest count of trypanosomes found in 50 fields of a blood smear was over 2,400. In the gland smear made at the same time a careful search failed to reveal a single trypanosome.

Conclusions.

1. *Trypanosoma congolense* was detected in blood smears of bovines as early as the fourth day after subcutaneous inoculation of infected guinea pig blood. In the gland smears the trypanosomes were first found on the ninth day.

2. *Trypanosoma vivax* was detected in gland smears of bovines on the sixth day after subcutaneous inoculation of blood of an infected bovine. In the blood smears the trypanosomes were first found on the fourteenth day.

3. In *T. congolense* infection the smear diagnosis is centred on blood smear examination, whereas in *T. vivax* infection, it is centred on gland smear examination, the gland and blood smear examination respectively, being of practically no assistance.

LITERATURE.

DU TOIT, P. J. (1929). A Note on the Diagnosis of *Trypanosoma vivax*. *15th Annual Report of the Director of Veterinary Services*, Onderstepoort, Pretoria, Part I, pp. 3-8.

Section II.

Virus Diseases.

R. A. Alexander	Heartwater. The Present State of our Knowledge of the Disease.
P. J. du Toit and R. A. Alexander	An Attempt to Attenuate the "Virus" of Heartwater by Passage.
C. Jackson	The Microscopic Diagnosis of Heartwater: A Preliminary Note on the Value of Intima Smears.
J. D. W. A. Coles	A Rickettsia-like Organism in the Conjunctiva of Sheep.
J. D. W. A. Coles	An Unknown Intracellular Organism of the Conjunctival epithelium of Sheep.

Heartwater.—The Present State of Our Knowledge of the Disease.

By R. A. ALEXANDER, B.Sc. Agric., B.V.Sc., Veterinary Research Officer, Onderstepoort.

Definition.

Heartwater may be defined as a febrile septicaemic infectious disease of sheep, goats and cattle caused by *Rickettsia ruminantium*, and transmitted by ticks of the genus *Amblyomma*, chiefly *A. hebraeum*. The disease receives its name from the common pathological lesion in sheep and goats, namely, a well marked hydropericardium, though this is not a constant nor the most pronounced finding in cattle. Animals which recover do not harbour the virus in the blood, which is infective during, and for a short time after, the reaction. Recovery from a reaction results in a solid, but not absolute immunity.

Synonyms.—Black Gallsickness. Mad Gallsickness. Black Lung. Swart Galsiekte. Dronk Galsiekte. Malkopsiekte. Herzwasser.

History.

Possibly the first authentic reference to Heartwater in sheep on record was made by Mr. John Webb on 23rd February, 1877, when giving evidence before the Cattle Diseases Commission at Grahamstown.* Webb drew attention to the ravages of the disease amongst the small stock of the Eastern Province of the Cape of Good Hope from the time its importance to the industry had been appreciated; about the year 1858. At that time a single farmer lost between 2,000 and 3,000 sheep and goats in one season from a disease which was believed to be Heartwater. Webb definitely correlated the incidence and spread of the disease with the advent of the Bont tick *Amblyomma hebraeum*, which was first noticed in the Cape of Good Hope by Mr. William Bowker when he discovered a specimen on a cow which had been brought from Zululand about the year 1837. There appears to be no record of the prevalence of the tick and the presence of the disease in Natal and Zululand at this time.

It is of interest to note that Webb during the course of his evidence made the definite statement that he had noticed the identical disease amongst Bushbuck along the Fish River at Southey's Poort. This observation has not been confirmed. Hutcheon (1900)

(*) 1877 Report of Cattle Diseases Commission of C. of G. H. Appendix, p. 108. Saul Solomon & Co., Capetown.

accepted Webb's observations as correct, and considered that the disease was first observed in sheep and goats near the coast on the borders of the King Williamstown and Peddie Districts, from which centre it was spread gradually to all points of the compass as the tick was distributed either by the movement of game or the trekking of domestic animals.

At this time considerable confusion existed in the minds of farmers and also of veterinarians as to the nature and aetiology of the disease, one of the factors contributing to the confusion being the recognition of hydropericardium as a fairly constant finding in post-mortem examination of animals which had succumbed to heavy infestation with intestinal parasites, chiefly *Haemonchus contortus*. In 1900, however, Hutcheon in his annual report clearly recognized Heartwater as a specific disease, was able to refute the various aetiological theories that had been advanced, and, as a result of the work chiefly of Edington, Dixon and Lounsbury, was able to give a good preliminary account of the transmission.

In the history of the disease a point of interest is that a careful perusal of the literature has failed to reveal any expression of opinion by a competent authority as to whether Heartwater is a disease indigenous to South Africa. On the contrary Lounsbury (1904) expresses the opinion that since Persian sheep, soon after their first importation into South Africa in 1872, were found to possess a strong natural resistance or insusceptibility, the disease may have had its origin in Persia and possibly other eastern countries, being introduced from there via the east coast of Africa. It is fairly certain, however, that the disease had been definitely recognized in the Cape prior to the first importation of Persian sheep and that its gradual spread resulted from the dissemination of infective ticks. But, it is not possible to express any opinion as to whether the spread of the disease was due to the importation of the virus, the vector having established itself previously, or to the importation of the virus in the tick, since our present knowledge makes it clear that the virus could not have maintained its existence in the absence of *A. hebraeum*.

Occurrence.

The disease has been reported from the Mandated Territory of South West Africa (Rickman 1908), Angola, French West Africa, and the Belgian Congo (van Saceghem 1918) and from Kenya and Tanganyika territorities. In 1925 Major Geoffroy and Dr. Poisson of the Service Veterinaire, Madagascar, had reason to believe, from the clinical symptoms and post-mortem appearances, that Heartwater was a cause of mortality in stock in Madagascar. Portions of kidney and cerebral cortex were forwarded to Onderstepoort, and these on histological examination revealed the presence of *Rickettsias* indistinguishable from *R. ruminantium* (Cowdry). In the Union of South Africa the exact distribution is obscure. The vigorous policy of tick eradication enforced by the Veterinary Department under the East Coast Fever Regulations, has resulted in the complete elimination of the disease from areas where previously it had been rife, and, in addition, the difficulty of obtaining a reliable diagnosis in suspected cases, prevents any accurate survey. Consequently, it must be assumed that the infected or potentially infected areas are all those where the

climatic conditions are favourable for the development of ticks of the genus Amblyomma. This includes the south and south-eastern portions of the Cape Colony characterised by summer rainfall, and excluding the Karroo; the whole of Natal and Zululand; the eastern and north eastern portion of the Transvaal, more particularly the area north of the Magaliesberg Mountains. The western, south-western portion of the Transvaal, and the Orange Free State appear to be free.

Animals Affected.

The disease was originally described in sheep and goats and, for many years, it was generally accepted that cattle were not susceptible. Lounsbury (1902) definitely showed that it was possible easily to infect cattle with strains of the virus obtained from either sheep or goats, and to-day it is generally recognized that the disease in ovines and bovines is identical, although the relative susceptibility of the different species varies within fairly wide limits. There are no data to indicate the insusceptibility or natural immunity of the various wild antelopes, and in spite of Webb's definite statement to the contrary, (*vide supra*), it must be concluded from general observations, that Heartwater is not a cause of mortality amongst game, though due regard must be paid to the possibility of these animals acting as a source of infection for ticks.

The early history of sheep farming in the Cape clearly established the fact that Persian sheep have a strong natural resistance. That they are resistant and not insusceptible was proved by Lounsbury and Robertson (1904), who found that a mild form of the disease was initiated by infection, from which the animals made a rapid and uncomplicated recovery with few clinical symptoms other than those of a slight fever. In addition, they found that the virulence of the virus for goats and Merino sheep, as demonstrated by subinoculation or transmission by ticks, was not modified by passage through numerous generations of Persians. This natural resistance is well developed in cross bred as well as in pure bred Persian sheep, and is also a pronounced feature of the Africander breeds.

Goats under natural conditions appear to be as susceptible as Merinos. In fact, transmission experiments under laboratory conditions have indicated that the mortality amongst goats is often somewhat higher than that amongst Merino sheep. This observation applies particularly to Angora goats.

The morbidity in cattle is often supposed to be low, even though they are known to be as susceptible as sheep and goats. This erroneous conclusion is probably due to the fact that native stock together with the grades obtained by mating with one of the improved European breeds, possess a variable resistance, which results in the disease frequently escaping recognition. The imported breeds of cattle are fully susceptible.

As regards the susceptibility of other animals, D. T. Mitchell in some unpublished work (Experiment S. 410 of 13.5.19, see Appendix page 134) has left records of an experiment in which a strain of Heartwater virus was apparently maintained, and propagated during passage through 8 generations of dogs, finally giving a distinct reaction in one of two subinoculated sheep. The same and other

investigators, together with the author, have attempted to repeat the experiment on several occasions under identical conditions, without any success. In view of this lack of confirmation therefore, it must be concluded that the virus will neither retain its infectivity nor multiply on passage through dogs.

Similar work has been carried out with rats and guinea pigs, the injections being made subcutaneously and intraperitoneally, with rabbits and horses injections being made subcutaneously and intravenously, without any evidence of infection. Consequently, the definite conclusion seems justified that the virus is specific for ovines and bovines and that small laboratory animals, the dog and the horse are neither susceptible to the disease nor are capable of serving as a source of infection for ticks under natural conditions.

At this stage it is necessary to direct attention to the work of Max Theiler (1930), who has shown that the virus of Yellow Fever may be maintained and ostensibly propagated by intracerebral injection through a large, possibly indefinite, number of generations of rats. It is not unreasonable to suggest that in Heartwater, where nervous symptoms in all probability of central origin, are so prominent a feature of the symptomatology, a similar method of infection of small animals may be successful. The value of such a means of retaining any particular strain of virus would be enormous in all experimental work, and it is hoped to remedy the omission by publishing a report on this aspect of the problem in the near future.

Aetiology.

Prior to the year 1898 various hypotheses were advanced as to the cause of the disease. Borthwick (1899) distinguished between Heartwater due to heavy infestation with helminths and specific Heartwater, but it was left to Hutcheon (1900) in his Annual Report to detail and refute the various theories that had been advanced, namely, the influence of climatic and " malarial " factors, overstocking, grazing on " sour " veld, calcium and phosphorus deficiencies. He concluded " that Heartwater has all the characteristics of a special or specific disease, that is, a disease which owes its origin to some living micro-organism which grows and multiplies in the animal body producing the pathological changes which are witnessed during the attack." This conclusion was based largely upon the work of Edington (1898) and Dixon (1899), who demonstrated that the disease was transmitted by the subinoculation of virulent blood, and Lounsbury (1900) who definitely established the transmission by *Amblyomma hebraeum*. This and subsequent work led Spreull (1904) to define Heartwater as " a specific febrile disease affecting sheep, goats and cattle in South Africa due to an ultravisible virus."

That an ultravisible virus was the cause of the disease was generally accepted, until Cowdry (1926) clearly demonstrated the causative relationship between *Rickettsia ruminantium* and the incidence of the disease, basing his findings upon—

(1) The discovery of *Rickettsia ruminantium* in the tissues of goats, sheep and cattle suffering from Heartwater; the association between their presence in these three species

and the febrile reaction; their disappearance soon after the fever has commenced to decline, corresponding to the loss in infectivity of the blood, and their absence in control animals;

(2) the appearance of the same *Rickettsia* easily identifiable by their very characteristic morphology and staining reactions in two series of ticks which had fed upon cases of Heartwater and which, before feeding, did not contain them; their non-appearance in three series of control ticks from the same point stock which had fed upon normal animals;

(3) the fact that the ticks containing *Rickettsia* in their alimentary tracts, when fed upon susceptible animals, produced in them typical attacks of Heartwater which the control ticks devoid of *Rickettsia* failed to do;

(4) the completion of the cycle by the recognition of the same *Rickettsia* in the tissues of these animals which contracted Heartwater as a result of having been bitten by the infective ticks containing *Rickettsia*.

Consequently, due credence must be placed upon the aetiological significance of *Rickettsia ruminantium*, though further investigation is necessary to clear up the relationship between the apparently ultravisible infective virus, as contained in the circulating blood, and the *Rickettsia*, as demonstrated in the tissues of affected animals and infective ticks.

In this article the term virus is used in the broadest sense to designate the infective agent in the animal or in the tick irrespective of its actual identity.

Rickettsia ruminantium (Cowdry 1926). It is not the intention to reproduce in any great detail the admirable description given by Cowdry in the three contributions made by the South African Expedition of the Rockefeller Institute for Medical Research (1926). These articles are of exceptional value in that they do not give merely an accurate description of *R. ruminantium*, but great care is taken to correlate the organism in the mammalian and arachnid host and to differentiate structures which may be confused. However, for the sake of completeness a " precis " of Cowdry's description is included.

R. ruminantium as seen in sections of tissue of the mammalian host is a very uniform spherical coccus shaped body 0.2 to 0.5μ in diameter. In a single clump the micro-organisms appear to be of the same size, but in adjacent clumps in the same section differences in size occur within the limits specified above. In small masses the size appears to be somewhat larger than in lightly packed larger masses, but the difference is not marked. Occasionally diplo-formation is observed, but this is the exception rather than the rule. No morphological evidence of a multiplicative phase has been observed other than this diplo-formation, which indicates the likelihood of simple division. The shape and size remain constant throughout the febrile reaction.

Stained with Giemsa the organisms are deep clear blue. Those in diplo-formation show no trace of red staining material between the

two halves as has been described in *Rickettsia* in lice, but sometimes fairly clear halos are visible. They are likewise easily stained by Löfflers methylene blue and other basic aniline dyes. When treated with Unnu-Pappenheims methyl-green pyronin mixture they usually stain light red, but sometimes take on a slightly greenish tint. They are Gram negative and stain readily with Fuchsin which is not retained on differentiation. They are easily stained with iron hamatoxylin, but on differentiation became bleached before the nuclear chromatin and the erythrocytes. With progressive autolysis of the tissue the affinity for basic dyes is lost before that of the nuclear chromatin.

In air dried smears of infective ticks fixed in absolute alcohol and stained with Giemsa, when closely crowded together the *Rickettsia* have the appearance of minute cocci about 0.2 to 0.3μ in diameter. When, however, the optical effect of superposition is avoided some appear to be bacillary in shape and others diplococcal. No filamentous forms have been observed. The bacilli measure 0.2 to 0.3μ by 0.4 to 0.5μ the pairs of diplococci 0.2 to 0.8μ. Stained with Giemsa they are usually coloured blue or light purple and in places where individual micro-organisms have been separated, many appear to be composed of two materials one coloured red and the other pale blue. The red staining material is present in two minute spherules embedded in the blue staining substance intervening between them, this substance often having a greater diameter than the spherules. The distance between the spherules is generally greater than that between pairs of diplococci. This dual staining property observed in smears is not seen in sections where the *Rickettsia* are invariably stained only one colour.

It is worthy of note that the staining reactions are more dependent upon the fixative and its mordaunting action than upon the stain. For example, with Giemsa they are coloured clear blue after Zenker's fluid, with or without acetic acid; bluish purple after Regaud's fluid, formalin or Carnoy's fluid; pale green after Flemming's fluid; and light pink after the picrosulphuric mixture of Mayer. In stained preparations the outlines are somewhat less distinct than bacteria.

The *Rickettsia* in the tick appear to be strictly limited to the epithelial cells of the intestine and to the lumen of the gut. It is probable, therefore, that under natural conditions infection takes place either by regurgitation or by the excretion of infective material from the gut.

Transmission of Heartwater.

A. Artificial.

In sheep and goats the disease is easily transmitted to susceptible animals by the intravenous injection of fresh virulent blood, either citrated or defibrinated, obtained from a reacting donor. Infection of cattle, either from other cattle, or from sheep or goats, does not appear to take place so readily or with such a degree of certainty. This observation may, in part, explain the original belief of the insusceptibility of cattle, and it has been brought out repeatedly when attempts have been made to infect comparatively large numbers of fully susceptible bovines at the same time in experimental work.

The intravenous injection of even 10 c.c. of virulent blood cannot be relied upon to set up the disease with certainty in all receivers, even though the susceptibility of the non-reactors may be established fully by a severe reaction to a subsequent injection. In one experiment 3 out of 12 susceptible yearling tollies failed to react to the same injection, and of the remaining 9 the reaction varied from a slight fever with no definite clinical symptoms to a severe attack with a fatal termination 3 days after the initial rise in temperature. Although the same experience has been encountered in sheep and goats, it is by no means so marked; less than 2 per cent. of animals fail to react, but at the same time non-reaction cannot be considered conclusive proof of insusceptibility. In experimental transmission work, therefore, it is essential to duplicate all injections. It is not possible to offer any conclusive explanation for this finding, but experience would seem to indicate that the variable factor is not the susceptibility of the receivers, but is the infectivity or virus content of the blood. A tentative assumption is advanced that the virus is not evenly distributed through the blood.

In all transmission work the method of subinoculation is of considerable importance. Intravenous injection, though not entirely reliable as indicated above, is the most dependable method. Of the other routes tried it is again not possible to state with any certainty the percentage infections which may be anticipated, but the following conclusions are justifiable:—

(*a*) subcutaneously more than 25 per cent. positive reactions cannot be expected (Experiment S. 2411, 1 positive out of 11).

(*b*) Intraperitoneally it is exceptional to set up a reaction.

(*c*) Intratracheally transmission has not succeeded.

(*d*) *Per os* transmission has not succeeded.

(*e*) Intradermally transmission has not succeeded though the infective dose was of necessity exceptionally small.

No data are available as to the possibility of successful transmission by intracerebral or subdural injection. This is an important omission which should be rectified at an early date.

The quantity of virulent blood subinoculated appears to be of some importance in that there appears to be a minimum amount below which it is inadvisable to descend if the result is to be relied upon. This statement is made in spite of the fact that fatal cases of Heartwater have been produced by the intrajugular injection of as small a volume as 0.1 c.c., in transmission work with at least three different strains of the virus. Experience, however, has indicated clearly that comparatively large quantities of blood must be used if the results are not to be discounted considerably. In all recent experiments an arbitrary standard has been adopted of 5 c.c. for ovines and 10 c.c. for bovines of fresh citrated or defibrinated blood and the results appear to have been entirely satisfactory.

The virus in the blood.—During the incubation period of the disease the blood is not infective and even the subinoculation of large amounts will not set up a reaction. Approximately 24 hours before

the initial rise in temperature the virus may be demonstrated in the peripheral circulation; there appears to be no difference in the severity of the disease set up by blood tapped at this stage, at the height of the thermal reaction or immediately before the temperature has returned to normal.

Animals which recover do not retain the virus in the blood stream. From the numerous experiments which have been undertaken to throw light on this point, it is apparent that individual variation is marked. In some cases no virus could be detected by the intrajugular subinoculation of 10 c.c. of blood 8 days after the height of the fever reaction, while in other cases a positive reaction was set up 35 days after the reaction but never after 45 days. On an average it may be stated that in the majority of cases the virus had completely disappeared 17 days after the return of the temperature to normal. As a general rule the severity of the reaction tends to decrease progressively with the gradual disappearance of the virus from the blood varying often from a rapidly fatal attack to one characterized by a slight febrile disturbance with no appreciable clinical symptoms.

The blood of an animal from which the virus has disappeared does not regain its virulence following one or more subsequent reinfections with the same or a different strain of virus unless a definite febrile reaction has been set up. When this occurs the virus tends to disappear more rapidly than during the previous sterilization.

At this stage it is of interest to note that the disappearance of the virus from the blood was demonstrated both by the intrajugular subinoculation of blood into susceptible sheep and by the attempted infection of larval *A. hebraeum* at various stages before, during and after the reaction. It also corresponds with the disappearance of demonstrable *Rickettsia* from the tissues.

The virus as contained in the blood has exceedingly limited keeping qualities. In carefully defibrinated blood stored at room temperature for 38 hours, the virus has been shown to be active in 5 c.c. amounts, but on no occasion has it been possible to set up a reaction after storage for 72 hours. In the majority of cases it has definitely been shown that the virus will not survive storage for 24 hours and even after 12 hours numerous cases are on record where the virulence had been completely or partially lost. Storage at low temperatures appears to have a favourable influence on the viability, but no data are available on the effect of temperatures below that of freezing.

As the poor keeping quality of the virus is a serious handicap in all experimental work, numerous attempts at preservation have been made without any success. These may be enumerated as follows:—

(*a*) The addition of 5 per cent. or 50 per cent. glycerine to blood destroyed the virus within 9 hours.

(*b*) Bleeding into a calcium oxalate-phenol-glycerine mixture (OCG Mixture) destroyed the virus within 12 hours.

(*c*) The addition of 1, 2, or 5 c.c. of 2.8 saccharose solution failed to preserve the virus in 5 c.c. of defibrinated blood for 8 days at 28° C.

(*d*) The subinoculation of 5 c.c. of defibrinated blood to which 0.1 per cent. or 0.05 per cent. glucose had been added failed to produce a reaction after storage for 7 days at 3° C., either aerobically or anaerobically.

(*e*) After 7 days storage no reaction was obtained with 2 c.c. of gently defibrinated blood to which had been added—

(1) liquid paraffin (kept at room temperature);

(2) 2 per cent. formalin (kept at 0° C.;

(3) isotonic sodium citrate (kept at room temperature);

(4) isotonic Locke-gelatine-dextrose (kept at room temperature);

(5) isotonic dextrose (kept at room temperature).

(*f*) The virus in defibrinated or citrated blood was not active after desiccation *in vacuo* over concentrated sulphuric acid the process of dessication having taken approximately 16 hours at room temperature.

(*g*) Dessication at 37° C. *in vacuo* in a "Schaerer" Serum Drying Apparatus over anhydrous calcium chloride failed to have any preservative effect.

As a result of the failure to devise any method of preserving the infectivity of virulent blood it became clear that the retention of any particular strain could only be accomplished in one of the following ways:—

(*a*) Repeated subinoculation into susceptible animals immediately after the height of the febrile reaction.

(*b*) Feeding either larval or nymphal *A. hebraeum* on the animal during the reaction and subsequently transferring the infection to the vertebrate host by either feeding the subsequent stage on a susceptible animal and/or injecting an emulsion of the activated tick virus (*vide infra*).

Localisation and Concentration of the Virus.—Repeated attempts have been made to ascertain the localisation and concentration of the virus in the blood of a reacting animal. The results obtained are not entirely satisfactory owing to fragile nature and poor keeping qualities of the virus, factors which always arouse the suspicion that, though the virus might have been present, it was so damaged during the processes of separating the various constituents of the blood that its presence could not be demonstrated by subinoculation into susceptible sheep. It may be concluded, however, that—

(1) the virus is not present in the serum or plasma obtained by prolonged centrifuging of either defibrinated or citrated blood;

(2) There appears to be no difference in the concentration of the virus in the erythrocytes or leucocytes obtained either by centrifuging or filtration through non-absorbent cotton wool;[1]

[1] Hornby. Observations on Rinderpest. *Jnl. of Comp. Path. and Therapeutics*, Vol. 41, No. 1.

(3) the virus appears to be attached to the erythrocytes and leucocytes in such a manner that it cannot be removed by repeated washing in isotonic saline solution.

In addition to the detection of the virus in the various constituents of the blood, its presence may be demonstrated in any organ which has a fairly rich blood supply, e.g. liver, kidney, spleen, though no attempt has been made to determine the relative concentration in those organs where *Rickettsias* are found most frequently on histological examination.

Variable results have been obtained with the injection of the serous fluid frequently encountered in the pericardial sac, and the peritoneal and plural cavities. It would appear that the infectivity of this fluid is dependent upon the presence or absence of erythrocytes and leucocytes or their disintegrated remnants which cannot be removed by centrifuging. Milk from lactating animals during the reaction is infective (Spreull).

Filtration of the Virus.—The difficulties attendant upon attempts to draw definite conclusions from the results of filtration experiments are too well known to require any elaboration here. Suffice it to say that many attempts have been made to demonstrate the presence of the virus by the injection into susceptible animals of the filtrate obtained after passage of blood through Berkefeld candles and Seitz filters, without success. Blood taken at the height of the reaction from naturally and artificially infected sheep and goats was diluted with 2, 3, and 4 times its volume of normal saline before filtration through Berkefeld W candles. Intrajugular injection of 10 c.c. of the filtrate into susceptible sheep invariably produced a slight thermal disturbance about the period corresponding to the reaction in the control animals, but at no time could a clinical diagnosis of Heartwater be made or confirmed by further subinoculations. Consequently it must be concluded that the virus will not pass a Berkefeld W candle when diluted 2, 3 or 4 times with saline, though no information is available as to the influence of alterations in Ph, electrical charge, etc.

With the Seitz filter identical results have been obtained. A definite reaction could never be produced with the filtrate of defibrinated or citrated, haemolysed or unhaemolysed blood, so that again it must be concluded that the virus cannot be aspirated through a Seitz filter.

Control experiments were conducted with the fluid obtained after aspiration through a thin layer of paper pulp. The virus undoubtedly passed such a barrier but subinoculation of varying dilutions indicated that it was present in the filtrate in an appreciably lower concentration.

Attenuation of the Virus.—The results of diagnostic subinoculations with fresh defibrinated and citrated blood has indicated conclusively that there is a considerable variation in the virulence of different strains of the virus and also of the same strain at different times. Whatever the reason may be, for instance plurality of the virus strains, influence of climatic factors, etc., every attempt to attenuate a virulent virus to a constant level under laboratory conditions has been unsuccessful.

There appears to be no progressive reduction of virulence on storing. Either the virus is infective and produces the normal reaction in susceptible animals commensurate with the extreme variability of reaction in all animals, or the virus is inactive. There appears to be no intermediary stage where there is a decrease in virulence preceding loss of infectivity. Similarly attempts at attenuation by rapid passage through successive generations of susceptible animals—a method which has proved entirely successful in the case of the virus of Blue Tongue in sheep—have yielded entirely negative results. In a few words it may be stated that the virus tends to decrease in virulence from time to time, but this decrease is subject to extreme variation, is not constant, and may be followed by a subsequent period of enhanced virulence for no apparent reason. At all events a constant and reliable attenuation cannot be relied upon by this method.

Attenuation by chemical, physical or thermal means *in vitro* have yielded equally disappointing results. Such methods offer no hope of success since the longevity of the virus is so distinctly limited.

Cultivation of the Virus in vitro.—Any attempts made have been entirely unsuccessful. Not only has it been impossible to propagate the virus but no methods of simple storage has been devised.

B. Natural Transmission of Heartwater.

It must be borne in mind that close and careful field observations by Dixon, Hutcheon, Edington, Spreull and others established a definite correlation between the presence of the Bont tick and the incidence of Heartwater soon after the disease had made its appearance in the Cape Province, but it was left to Lounsbury to clear up the problem. His work, which was published in 1901, was the outcome of carefully planned and conducted research experiments. The final conclusions stand practically unmodified and with few additions to-day.

In his report for 1901 in his capacity as Government Entomologist, Lounsbury draws the following conclusions (some of the findings are hardly relevant to this chapter but they are included for the sake of completeness):—

1. *Amblyomma hebraeum* is normally non-pathogenic for ovines and bovines.
2. Infection is transmitted by nymphae which were fed as larvae on sick animals.
3. Infection is transmitted by adults which are fed as nymphae on sick animals.
4. Infection is transmitted by adults fed on a cow as nymphae but on sick animals as larvae.

   Note.—At this time the identity of the disease in sheep, goats and cattle was not admitted; in fact, cattle were regarded as insusceptible. Lounsbury, however, definitely establishes the principle of non-cleansing of the infective tick.
5. Infection is transmitted by adults fed on a long-recovered goat as nymphae but on a sick animal as larvae.

6. Infection is transmitted by nymphae fed as larvae on a recently recovered animal; negative when fed 35 days after recovery though positive 14 days after recovery.
7. Infection is transmitted to recovered animals by pathogenic nymphae.
8. The progeny of pathogenic Bont ticks are non-infective.
9. Male *Amblyomma hebraeum*, transferred from a sick to a healthy animal, appear to be innocuous.
10. Bont ticks dropped durnig most of the incubation period are innocuous.
11. Bont nymphae from long recovered animals either transmit only very mild infection or none at all.
12. *Rhipicephalus evertsi* are not vectors.
13. *Boophilus decoloratus* are not vectors.
14. The Tampan (*Ornithodorus savignyi*) is not a vector.

At one time or another all the points brought out by Lounsbury have been confirmed. During the course of this confirmatory work the following additional information has been made available:—

1. *Amblyomma* males fed on a sick animal as nymphae are capable of transmiting the disease either by feeding or by the injection of the emulsified tick after activation of the virus (see *infra*).
2. *A. hebraeum* adults that fed on an insusceptible or susceptible animal as nymphae but on a reacting animal as larvae are capable of transmitting the disease.
3. *A. hebraeum* nymphae fed on a reacting animal as larvae transmit the infection after feeding for 60 hours and probably after feeding for 48 hours.
4. *A. marmoreum* and *A. nuttalli* do not carry the infection from larvae to nymphae. The author has not succeeded in feeding the adults of either of these species on susceptible animals.
5. *A. variegatum* is capable of transmitting the infection from nymph to adult (Daubney 1930). Neither the transmission from larvae to nymphae nor the principle of non-cleansing when fed on a susceptible or insusceptible host has been marked out for this species.

The above summary indicates that, in South Africa at least, the chief vector of Heartwater is *A. hebraeum*. It is not the intention in this article to enter in any great detail into the life cycle of this tick, but certain aspects of its biology are so important, both for a clear conception of the disease and, as an aid to other investigators that a short description mainly based on the confirmed findings of Lounsbury will not be out of place.

The species appears to be able to subsist on any warm-blooded animal and either has been fed or has been found attached in all its stages on the horse, donkey, mule, sheep, goat, dog, ostrich, fowl,

antelopes of several species and man. The ox, however, is its principal host and the one to which it is best adapted. As a larva or nymph it freely attacks any part of the body, but as an adult it commonly does not attach high on the flanks, along the back or about the head. Eggs maintained in the laboratory at room temperature have hatched in from 24-226 days with an average of about 96 days. Under natural conditions eggs laid in the spring and early summer generally hatch before the cold weather, but those laid in the late summer and during the winter usually remain unhatched until midsummer the following year. The shortest adult larval stage at ordinary temperatures observed was 26 days, the longest 279 days. The time required for engorgement of the larva on the host varies slightly with the species of animal, and more with the portion of the animal to which it is attached, presumably owing to the greater ease with which blood is drawn from one place than another. The stay is usually 5-6 days, but may be as short as 4 and as long as 20. The larvae nymph period under laboratory conditions is usually 30 days, but may vary from 17-126 days. The nymphae usually take 12-24 hours longer to feed than the larvae. The nymph adult period varies from 10-160 days with an average of 65 days. The adult male takes up a position on an animal and may remain many months—even up to a year. In about five days he is ready to mate and exhibits his inclination by erecting his body and waving his limbs should a female approach him. The female is loth to attack except in the immediate vicinity of a sexually mature male and she appears to delay final engorgement until fertilized. This point is of extreme importance in any experimental feeding operations with adult *A. hebraeum*. Portion of this work was considerably hampered and delayed by the failure of several batches of adult female ticks to attach and engorge on the tick feeder. Following the observation that engorgement proceeded rapidly when males had been placed on the host about seven days previously the difficulty was overcome. After finding a mate the adult female usually takes about six days to feed to repletion though the period may be prolonged to about fifteen days.

For optimum development *A. hebraeum* prefers a warm but only moderately moist climate; in all experimental work this preference must be borne in mind since heavy mortality in batches of ticks may occur if the atmosphere of the containers is allowed to become saturated with moisture; development will be unduly prolonged if the temperature falls below 20° C. The tick thrives best in veld shaded by high bushes and quite fails to establish itself in open low grassveld and in the Karroo. Its occurrence in the Cape Province is restricted to the southern and south-eastern districts characterised by a summer rainfall. Shade appears to be necessary to prevent dessication of the quiescent stages, but at the same time a prolonged excess of moisture has a distinct unfavourable influence on development.

The species is characterised by fairly considerable longevity. It is common to find unfed larvae and nymphae alive and vigorous after 6 and 8 months, though all the ticks in a breeding tube may succumb in as short a period as 30 days. Individual adults have survived for 15 months. Under laboratory conditions, it is not advisable to attempt to feed the ticks until at least a fortnight after hatching or moulting.

With the death of the host a small percentage of attached unengorged individuals will detach and will feed to repletion on another animal subsequently; the majority, however, succumb.

Amblyomma marmoreum and the closely allied *A. nuttalli* in their general life history, resemble *A. hebraeum*, except that the period of engorgement of the adults which have only been fed on cold-blooded animals, is usually prolonged to six weeks or more. Insufficient data is available to give figures of the length of time commonly taken for completion of the various stages. The periods obtained from the limited number of batches that have been reared might be misleading. A point of interest is that it has not been found possible to feed adults of either of these species on warm-blooded animals. A tortoise has been found admirably suited for the purpose. The larval and nymphal stages are easily fed on sheep, goats and cattle.

Amblyomma variegatum is encountered chiefly in Rhodesia, the central African States and Madagascar; it is not found in South Africa. The same difficulty in rearing this species has been encountered, as recorded by other investigators (Lounsbury, Daubney). On two occasions the entire progeny of one female was reduced to a single individual by the time the adult stage had been reached. The reason for this is quite obscure, but it seems to stand in some relation to moisture and temperature in which requirements the species is particularly exacting.

C. Transmission by the Intravenous Injection of the Emulsified Infective Invertebrate Host.

Prompted by the results obtained by Spencer and Parker (1924 *et seq.*), working with Rocky Mountain Spotted Fever, Theiler and du Toit (1928) attempted the transmission of Heartwater by the injection of emulsified ticks. They concluded that the intrajugular injection of emulsified infected *A. herbraeum* fed nymphae produced the disease in the vast majority of instances (8 out of 9), while the injection of similar unfed nymphae succeeded rarely (in one instance when the course of the disease was considerably milder than that in controls). The minimal infective quantity was that containing 0.1 c.c. tick. The emulsion was infective when prepared by grinding in saline with or without the medium of sand, filtered or unfiltered. When mixed with 50 per cent. glycerine-saline its virulence was destroyed. The feeding of the nymphae apparently increased the virulence of the virus. Attempts to activate the virus by incubation of the ticks at 37° C. in place of feeding were negative.

During the course of various attempts to develop a method of immunisation by formalisation of the activated tick virus in a manner analagous to that reported by Spencer (1929) against Rocky Mountain Spotted Fever, the author was able to repeat and confirm portion of the work of Theiler and du Toit. No attempt was made to ascertain the infectivitiy of emulsified unfed or incubated nymphae which had picked up their infection by feeding as larvae on animals during a Heartwater reaction. It was, however, confirmed that similar fed nymphae emulsified after feeding to repletion were capable of producing a virulent form of the disease when injected intrajugularly into susceptible sheep and cattle, whether the ticks had transmitted

the infection to the vertebrate host during feeding or not. Infection was produced in less than 50 per cent. of cases. In one instance infected nymphae which were forcibly removed before full engorgement at the 48th and 60th hour, transmitted Heartwater to the bovine on which they had been attached, but on emulsification it was not possible to demonstrate the presence of any virulent virus by subinoculation. Concurrently it was definitely shown that the virus, activated by feeding on a susceptible animal, was no more stable and possessed no better keeping qualities, than that contained in the blood of a reacting animal. One attempt to determine whether the virus could be activated by feeding on an insusceptible animal, e.g. the horse, gave a negative result. Similarly an attempt to produce the disease by intravenous injection of emulsified engorged adult ticks that had been fed as nymphae on a reacting animal, was unsuccessful.

It will be seen readily from the above results that there remains a considerable amount of work to be done on the virus in the tick. This work is considered to be of great importance because experience has clearly shown that the virus contained in the blood of a reacting animal is so inconstant in amount and infectivity, is exceedingly fragile with limited keeping qualities, that, if any method of active immunisation based in injection of a modified virus is to be elaborated, the most reliable source of that virus probably will be the activated infected tick either in the nymphal or adult stage.

Suggested points which require detailed investigation are:—

1. The reason why ticks of the same batch after being fed on the same or different animals do not always produce the disease after emulsifying.
2. Whether infected adults may be activated in the same way as nymphae.
3. Whether it is necessary to feed the ticks to repletion before activation of the virus can be relied upon. If not, what is the optimum period of feeding?
4. What are the changes that occur in the virus in the tick which render it virulent after being in contact with ingested blood? Should any morphological changes occur, does this represent a phase in the life cycle or development of the *Rickettsia*?
5. Will the addition of fresh defibrinated or citrated blood to emulsified infected ticks in vitro activate the virus with or without incubation?
6. When does the change to infectivity of the virus commence and when does it attain its maximum?

At this stage it is necessary to point out that the intrajugular injection of emulsified ticks is not unattended by grave danger to the recipient. With a quantity of emulsion containing five nymphae and 0.5 adult, immediate shock and death within a few minutes has resulted. The intensity of the shock produced decreased with the quantity of material injected and has not been observed with 1 nymph and 0.1 adult. The symptoms produced in sheep are trembling of the muscles, unsteady gait, polypnoea and extreme respiratory distress chiefly inspiratory in nature, accelerated pulse, general venous stasis

and profuse diarrhoea. In those cases which recovered the symptoms commenced to decline after approximately 30 minutes and had disappeared within three hours. In animals which died, a prominent feature of the post-mortem examination was a well-marked glottis oedema.

Symptoms.

The period of incubation varies considerably. Cases in sheep and goats are on record where the onset of the disease was noticed five days after the injection of virulent blood; on the other hand, an incubation period of as long as 35 days has been observed under conditions where accidental natural infection subsequent to injection could be excluded with a reasonable degree of certainty. The vast majority of cases, however, show the first symptoms of the disease between the 7th and 14th day after intravenous injection and then usually about the 9th day. As a general rule the period of incubation tends to vary inversely with the size of the infective dose, when amounts less than 5 c.c. are used.

In cattle the average incubation period is slightly longer than that in sheep and goats, being approximately 10-16 days with the majority of cases occurring round the 12th day.

After natural infection with ticks the periods in both ovines and bovines are lengthened by approximately two days, though extreme variations again are not uncommon.

As a general rule the period of incubation of any particular strain of virus is fairly constant as indicated by the incidence of the disease after successive repeated sub-inoculations, and the rise in temperature in a number of animals on the same day when extensive experiments were in progress. There appears to be no correlation between the length of the incubation period and the severity of or mortality from the subsequent attack.

Depending upon the susceptibility of the animals, the duration of the disease and the virulence of the virus several different forms of Heartwater may be differentiated:—

A. Peracute form;
B. Acute form;
C. Sub-acute or chronic form;
D. Mild abortive form or Heartwater fever.

A. By the *peracute* form is understood those cases characterised by sudden death with the presence of clinical symptoms for periods usually not longer than 36 hours. In the morning an affected animal may appear perfectly normal, feeding or ruminating and having an internal body temperature well within the limits of normal variation, yet within a few hours the temperature may rise to 105°–106° or higher followed by sudden collapse and death after a few paroxysmal convulsions. Symptoms when they are observed are those of severe fever followed by sudden collapse. Cases of this nature are encountered in either sheep, goats or cattle whether infection was by artificial or natural means and present considerable difficulty in diagnosis.

B. The *acute* form is by far the most common in all susceptible animals. Usually the first indication of any abnormality is a sudden rise in temperature up to 105.5° or more within 12 hours. Occasionally this rise in temperature may be more gradual, increasing by 2° per day until a maximum of say 108° is reached. This hyperthermia is usually the only initial demonstrable symptom, the animal frequently feeding and ruminating normally, though variable sluggishness may be noticed in animals at gross. The temperature remains high and fluctuations are slight. Gradually inappetence and cessation of rumination supervenes, the animal acquiring a tucked-up appearance, and an anxious staring expression of the eyes; in bovines the hairy coat is harsh and staring. It is noteworthy, however, that feeding frequently continues until a short time before collapse and death. Breathing is increased in frequency, being costo-abdominal in nature. The pulse is accelerated, the pressure and excursion remaining good, until the later stages, when it becomes weak and thready. The conjunctiva is reddened but extensive submucous haemorrhages are not seen usually. On percussion of the thorax it is seldom possible to determine the presence of any hydropericardium though hydrothorax of a variable degree may be established. Auscultation invariably reveals the presence of moist râles. Nervous symptoms gradually supervene with the progress of the disease. Sheep and goats are noticed to show a progressive unsteadiness of gait and commonly stand in an attitude with legs apart, head down, ears dropping and with flanks and chest heaving. In fatal cases they eventually go down and remain stretched out on the side, showing almost continuous galloping movements of the legs, masticating movements of the jaw, frothing at the mouth with continual licking of the lips, the head thrown back and strabismus. Later the paroxysms become intermittent, the respirations stertorous and grunting. Emaciation is neither particularly marked nor rapid. At this stage affected animals bear a close resemblance to those suffering from strychnine poisoning or tetanus, all the muscles of the body being tensed in response to any external stimulus. Agony may be prolonged up to 24 hours or longer.

The acute form of the disease usually has a duration of from three to six days. Animals which go down with marked nervous disturbances seldom recover. Those animals which recover show a gradual dimunition of the common symptoms, the temperature usually being the first to return to normal. Complete recovery is usually prolonged, and sheep usually shed the whole or the body portion of the fleece.

The symptoms in cattle are practically identical with those exhibited by sheep and goats, though disturbances of the nervous system are usually somewhat more pronounced in the stages before the animal goes down. First one may notice merely the twitching of individual muscles, the head being held in a dazed fashion with involuntary nodding or swaying movements and twitching of the ears. The unsteady gait becomes progressively more pronounced, affected animals walking with a marked swaying action of the hind-quarters, and stepping high with the fore legs, which are frequently crossed. Individuals often wander aimlessly in a circle and will be seen to bump into or stagger against objects as if the sight had been impaired.

Alternately affected animals may become vicious. In contradistinction to small animals diarrhoea is usually marked, being dark in colour, fluid and evil smelling. Polyuria but never haemoglobunuria may be noticed.

C. In the *sub-acute* or *chronic* form the symptoms closely resemble those described for the acute form but are not so intense or well marked. The temperature remains elevated and there is no tendency to marked remissions and exacerbations. Affected animals may linger for periods up to 10 days or more, when they may collapse and die suddenly, or the temperature may return to normal gradually and the symptoms become progressively less pronounced. Frequently death results from complications such as hypostatic pneumonia or as a sequel to prolonged atony of the fore stomachs. Complete recovery is slow.

D. The *mild abortive* form is characterised by an absence of all clinical symptoms other than a hyperthermia which is often marked. It is seen in animals which possess a strong natural resistance, e.g. Persians, or in those which have acquired a natural immunity by recovery from a previous attack. It is certain that under natural conditions many such cases completely escape detection, and are dangerous because they serve as a reservoir for the infection of ticks. Under laboratory conditions this abortive form is detected from the temperature charts, the diagnosis being confirmed by sub-inoculation when the typical acute form usually is produced. The temperature disturbance rarely lasts longer than two or three days and the general health of the animal does not appear to be affected in any way.

Morbidity.

Under laboratory conditions it is frequently observed that, when a large number of susceptible animals are injected with, say 5 c.c. of fresh citrated infective blood, all the inoculations being carried out under precisely similar conditions, and at the same time, one or more will fail to react, though their susceptibility may be established without doubt by subsequent severe reaction to a similar infective dose. The reason is quite obscure. Similarly in tick feeding work different ticks from the same breeding tube may produce Heartwater in one animal and not in another, even though the susceptibility of both is not open to question, the animals having been reared from birth under carefully-controlled tick-free conditions in tick-free stables. This occurs, in spite of the fact that a single tick is known to be capable of transmitting the disease. Again the reason is obscure, but an explanation can be sought in the finding that all ticks which feed on an animal passing through a Heartwater reaction, do not themselves pick up the infection. This is supported by the variation in frequency of *Rickettsias* or even their apparent absence in infected ticks from the same breeding tube.

Under farming conditions similar observations have been made. For instance, in fully susceptible cattle imported from the Orange Free State, where Heartwater does not occur, to the heavily infected areas of the Northern Transvaal, cases of the disease will continue to occur in different animals over a period of many months, though the entire herd may be running under identical conditions of heavy tick

infestation. The probability is, however, that on an infected farm all susceptible animals will contract the disease sooner or later though clinical manifestations may vary within wide limits.

Mortality.

The mortality varies considerably with the locality, i.e. with the strain of virus and on the same farm it varies from year to year and also in different seasons of the same year (c/f Epizootology). In laboratory experiments the mortality in Merino sheep has varied between the wide limits of 40-90 per cent. In one experiment (S. 4019), involving the infection of 162 Merino sheep with eight different strains of the virus over a period of 10 months, the mortality was 74 per cent. Under field conditions, a figure somewhat higher than this can be expected in sheep, in goats considerably higher. In fact, in all cases where a definite diagnosis may be hazarded, a distinctly adverse prognosis is warranted. As previously stated, Persian sheep usually suffer merely from Heartwater fever with a mortality less than 5 per cent., and the percentage deaths amongst cross-bred Persians is considerably less than that amongst Merinos. Amongst cattle the mortality fluctuates round an average of approximately 60 per cent. and a guarded prognosis should always be given.

Post-mortem Appearances.

The macroscopic pathological lesions have been described in great detail by Steck (1928). The author has little or nothing to add to or modify in this description, but the protocols of post-mortem examinations on a number of Heartwater cases considerably larger than those consulted by Steck have been examined.

In peracute cases it is generally quite impossible to venture any diagnosis from the post-mortem lesions, since there may be no deviation from normal in any respect. Usually there is a *tumor splenis* and a limited number of subepicardial and sub-endocardial haemorrhages.

The typical lesions which, however, cannot be considered pathognomonic are seen in the acute, sub-acute and chronic cases. It has been noticed that the predominant lesions vary with the strain of virus and are fairly constant for any particular strain. An additional point of interest is that complications do not occur frequently as in distemper in the dog, the majority of the lesions standing in direct relation to the disease. Two exceptions may be mentioned, the first being decubitus and skin abrasions due to prolonged prostration and injuries sustained during convulsions, the second pneumonia usually hypostatic in nature.

The cadaver is usually in good condition, and in acute cases it is found that feeding had continued until shortly before death. There is no change in the integument other than those lesions due to mechanical injury. Ticks may or may not be present; the absence of *A. hebraeum* has no significance, since the infective ticks would certainly have dropped during the incubation period, and in addition there is a tendency for unengorged Bont ticks to detach from the host during the agonal period. The visible mucous membranes may be injected though this is not a constant finding; similarly haemorrhages

are variable. There is no apparent alteration in the blood and flesh. The subcutaneous tissue is normal, except for evidences of mechanical trauma: it is noteworthy that in spite of extensive exudations into the serous cavities any trace of general subcutaneous infiltration or anasarca is not encountered. The peritoneal cavity usually contains an excess of fluid which may vary in amount from 20 c.c. to 1 litre in sheep, and several litres in bovines. The same alterations occur in the pleural cavity. The pericardial sac may or may not be distended with fluid, which is straw coloured, though occasionally it is blood-stained, clear and not viscid; it may contain small flocculi and may be partially coagulated, the coagulum then being pale in colour and soft. It is usually stated that this fluid coagulates promptly on exposure to air, but careful collection and observation of the exudate or transudate from a large number of undisputed cases of Heartwater in sheep, goats and cattle, has shown that in approximately 10 per cent. of cases no coagulation occurs. The presence of this pericardial fluid is a fairly constant finding in ovines, but in cattle, in the majority of cases, it is conspicuous by its absence. The myocardium, apart from the presence of sparsely distributed punctiform haemorrhages frequently shows evidence of cloudy swelling and fatty degeneration as indicated by pallor, opacity and decreased consistence. The serous membranes are smooth, glistening and transparent, though a variable number of small haemorrhages may be present. In connection with the respiratory tract there is usually a serous or mucous discharge from the nostrils. The pharyngeal, laryngeal, and tracheal mucosa is hyperaemic, covered with mucous and showing distinct ramiform injection and prominence of the lymph follicles; small haemorrhages may be present. In the lungs an almost constant finding is an extensive oedema, the bronchi and bronchioles containing large quantities of froth rarely blood-stained. The subplural, sublobar and peribronchial connective tissue is distended and clear. Small haemorrhages are frequently scattered through the parenchyma, and areas of atelectasis in the ventral portions of the lungs are common as a result of the hydrothorax. The liver is usually enlarged and intensely haemorrhagic as seen from the large quantity of blood-stained fluid which exudes from the cut surface. On section the central veins are distended, the periportal connective tissue frequently prominent, distended and clear, fatty degeneration and bile stasis marked. The distension of the gall bladder with bile has led to the common name "gallsickness". The kidneys are hyperaemic, the glomeruli being prominent and the intermediary zone deep reddish purple; variable fatty degeneration is present. The spleen almost invariably is enlarged, and this enlargement may be considerable up to 100 per cent. in bovines and 60 per cent. in ovines. On section the cut surface bulges, only a small amount of semi-fluid material can be scraped off, the Malpighian bodies and trabeculae are indistinct and the consistence is more pulpy than normal. The retropharyngeal, submaxillary, and peribronchial lymphatic glands are usually enlarged, haemorrhagic and exceedingly moist on section; the superficial lymphatic glands are not noticably enlarged. In the alimentary tract the first abnormality is encountered in the abomasum, in which the mucous membrane is much thickened and on section infiltrated with transparent fluid. The fundus and cardia show numerous minute haemorrhages, while the pylorus is diffusely reddened, often showing epithelial

desquamation and even ulceration, these changes being more prominent on the folds. The gastric changes are very much more pronounced both in intensity and frequency of occurrence in cattle than in sheep and goats. The small intestines show changes similar to those seen in the abomasum, being marked in the duodenum, decreasing in intensity and becoming patchy in the jejunum and increasing again towards the ileo-caecal valve. Frequently patches of ramiform injection and diffuse hyperaemia alternate with areas where nothing unusual can be detected, producing the so-called "Zebra markings." Again these changes are more noticeable in cattle. The large intestine is frequently normal in appearance, though hyperaemia decreasing in intensity caudally, may be evident. In the central nervous system there may be some increase in the amount of cerebo-spinal fluid, and the meninges are frequently hyperaemic. The urogenital organs and skeletal system show no deviation from normal.

Histo-pathology.

The only detailed histological study in cases of Heartwater published is that by Steck (1928). As the author has had no opportunity of carrying out any confirmatory or additional work on the subject a summary of Steck's findings is given here for the sake of completeness. He states "that besides the presence of *Rickettsia ruminantium* (Cowdry), the characteristic changes in Heartwater are leucostasis and perivascular cellulation. The former occurs in all organs, and the macrophage is the prominent cell, but lymphocytes and neutrophiles are also numerous. The latter is pronounced in liver, kidney and sometimes the adrenal glands. All the cells of the mesenchyma may take part in this reaction, but mainly those of the lymphocytic series: lymphocytes and plasma cells.

It seems most likely that these alterations are due to a noxe which is spread diffusely by the blood stream. It must remain for future investigation to determine the nature of the noxe."

A point of considerable interest is that both Cowdry and Steck are agreed that there is no local reaction to the presence of *Rickettsias*. Infected cells, except for the mechanical displacement of the nucleus, appear to be quite unaffected. In the kidney, for instance, the correlation between the interstitial changes and the presence of *Rickettsias* ceases when it is stated that in general *Rickettsias* are present in numbers more easily detected when the interstital changes are most pronounced. But *Rickettsias* may be numerous with only slight changes in the interstitium and vice versa. The local cellulation is not a local reaction to the presence of microscopically detectable *Richettsias*. These *Rickettsias* are most easily detected in the endothelial cells of the capillaries of the renal glomeruli and in the superficial grey matter of the cortex. They were aso found in the following tissues in order of frequency: spleen, lymph glands, corpus luteum, cerebellar cortex, suprarenals, midbrain, medulla oblongata, ovaries, corpus striatum, salivary glands, pancreas and heart muscle. They were never found in either the liver or the lungs.

Daubney (1930) states that after considerable experience of routine histological examination of the kidney and central nervous system in suspected cases of Heartwater, he is able to confirm the

findings of Steck and Cowdry. He directs attention to the observation, however, that certain lesions of the central nervous system were remarked as characteristic of the disease, and that these lesions are most pronounced in the bovine cases, a fact which agrees with the clinical observations. In addition to the leucostasis in sections of the cerebral or cerebellar cortex, and some evidence of perivascular infiltration and subarachnoidal cellular exudation, there occur with great regularity in bovines and frequently also in sheep the so-called "Sattelism" of murous. This sattelism is in every way comparable to that described by various authors (Nicolau and Galloway, 1928, and others) in Borna disease of the horse and by Metchnekoff in senility. To what the sattelism is due is not clear but apparently it is not a direct result of the presence of the *Rickettsias* but is produced as a reaction to the circulating virus.

Diagnosis.

At the outset it must be made clear that, up to the present, no completely satisfactory method of diagnosis has been elaborated. Consequently, it is certain that large numbers of animals, both large and small, succumb to Heartwater, without a definite aetiological diagnosis being made even by experienced veterinarians.

In typical cases in bovines the diagnosis may be fairly certain from a consideration of the clinical symptoms in conjunction with the post-mortem lesions; in sheep and goats from the post-mortem lesions considered in the light of an accurate anamnesis and clinical examination. Due regard must always be paid to the presence and identity of ticks on the animals in contact and the known occurrence of the disease in the neighbourhood. It has been pointed out, however, that cases typical in all their manifestations, are the exception rather than the rule, so that, after even a careful clinical and post-mortem examination, a suspicion of the aetiology rather than certainty is the finding.

Blood-smear examination is of no assistance. *Rickettsias* have never been observed either in stained blood smears, stained in a variety of ways at intervals during the febrile reaction and after death, nor in fresh blood either by direct or oblique illumination.

Histological examinations of sections of kidney and cerebral cortex has proved of value in the hands of Daubney and others, but it is a method which has not found favour and which has not been adopted as a routine measure in South Africa. The method is comparatively slow and would entail a considerable amount of work both in section cutting and examining, if a large number of specimens had to be handled. Moreover, the *Rickettsias* retain their staining properties for only a few hours after the death of the animal, though they have been identified with difficulty when specimens have been taken six hours after death and kept for a further period of $11\frac{1}{2}$ hours at room temperature (Cowdry). Under laboratory conditions, when suitable pathological specimens can be placed in fixative solution immediately after death, the method is undeniably of great value, but under South African field conditions, where autopsy is seldom held within 24 hours of death, and where suitable fixative is rarely available, histological examination as a means of diagnosis has and cannot be other than of little importance.

Jackson (1931) has worked out a method of diagnosis which has as its basis the constant finding of *Rickettsias* in the endothelial cells present in intima smears of the larger blood vessels. This method has a decided advantage over the histological examination of sections, in that it is quick, entails less labour and requires the possession of no special material by the collector. But again its practical application in the field is open to doubt, although the technique is simple, since the making of smears within a few hours of death is an essential. Moreover, the method is of no value for ante-mortem diagnosis, and is not applicable to those cases which recover from a febrile disease of unknown aetiology without exhibiting any pathognomonic symptoms.

Diagnostic subinoculation into susceptible small animals is a method which has proved of considerable value, but again only under laboratory conditions where the injections can be made rapidly before the virus has lost its infectivity. Since the virus in all the domesticated ruminants is identical cheap Merino culls have been used with success. The limitations of this method require no elaboration but briefly they may be enumerated as follows: —

1. The extreme slowness of the method not so much in clearly positive cases but in negative cases. In the latter it is not possible to express any opinion until after a period of four weeks, as the possibility always exists that the particular virus may be characterised by an unusually long incubation period.
2. The difficulty of interpreting a reaction in the subinoculated animals unless the reaction is fatal or very severe. In addition, the variable period of incubation makes it possible for febrile reactions other than those due to Heartwater to obscure the result, making further subinoculations and further delay necessary.
3. Except under carefully controlled conditions the susceptibility of the receivers may be open to doubt.
4. The uncertainty always exists that when the subinoculation gives a negative result, the case may have been one of Heartwater, failure to react being due to inability of the delicate virus to survive transportation over even comparatively short distances. In one instance subinoculations proved negative from a case of Heartwater, subsequently confirmed by detection of the presence of *Rickettsias*, after the blood had been conveyed 30 miles over a bad road by car and injected eight hours after withdrawal.
5. The expense of the method is considerable when compared with the cost of diagnostic methods employed in other diseases.

Of the serological methods, complement fixation has been of no value. It has not been possible to elaborate any method based upon an allergic reaction.

Differential Diagnosis.

Since no accurate method of making a rapid diagnosis of Heartwater in the living animal has been evolved, the question of differential diagnosis assumes increased importance. This importance will be

appreciated by the clinician who usually is in a position to hazard a diagnosis only when other diseases with a somewhat similar symptom complex have been excluded by a process of elimination. The following common conditions in ruminants must be borne in mind:—

1. Infestation with intestinal parasites. Prostration often with nervous symptoms is encountered frequently. Infected animals, however, are usually in a state of cachexia, and individuals in various stages of debility will be found in the flock or herd. A different anamnesis is obtainable, there is an absence of fever, the mucous membranes are usually pale, and an oedema of the intermandibular space is common. At post-mortem the parasites are found, tumor splenis is absent, pulmonary oedema is not so pronounced, the pericardial fluid shows no tendency to coagulation and the gastro enteritis, in cattle especially, is not so marked.
2. Blue Tongue in Sheep. This is a disease which may be confused, since seasonal occurrence and the geographical distribution is similar. The lameness due to specific pododermatitis, and the lesions of the buccal mucosa, are of importance. Emaciation is rapid. Frequently it is necesasry to resort to subinoculation into Heartwater-susceptible calves to furnish a diagnosis pending histological examination of sections.
3. Diseases caused by pathogenic protozoa, e.g. Redwater, East Coast fever, Anaplasmosis, may be confused in cattle, owing to the intestinal affections resulting in diarrhoea, the anaemia resulting in pronounced oedemata, and the frequent development of nervous symptoms. The latter, however, are more violent in nature and tend to make affected animals actively aggressive. Microscopic examination of suitably prepared blood smears is generally satisfactory.
4. Snotsiekte may be differentiated from the history, the rapid emaciation, the more profuse diarrhoea, the corneal opacity and the marked enlargement of the superficial lymphatic glands. The symptoms combined with the post-mortem findings are conclusive.
5. Malignant Catarrhal Fever bears a very close resemblance to Snotsiekte, and is rarely transmissible by the subinoculation of blood. Diphtheritic lesions of the visible mucous membranes of the alimentary and urogential tracts are a marked feature.
6. Rinderpest. In the days before the specific identity of Heartwater had been established, and when the immunisation of cattle against Rinderpest by the simultaneous serum virus method was being practised, this disease had to be taken into serious consideration from a differential diagnostic point of view. It must still be of importance in those areas where Rinderpest is enzootic. The different course of the disease, the direct transmission and the characteristic lesions of the Peyers patches, however, are diagnostic.

7. Subacute cases of Heartwater where prostration and nervous lesions are pronounced, had a strong resemblance to Tetanus or Strychnine poisoning. The anamnesis is usually conclusive and in Tetanus the muscular response to external stimuli is more marked.
8. Similarly both peracute and subacute cases of Lamsiekte must be borne in mind. The differential diagnosis does not present great difficulty, but undoubtedly frequent confusion has occurred and still does occur.
9. Typical cases of Diploidia poisoning require mention, though the history of the incidence of the disease usually in the latter part of the winter, after animals have been grazing in old mealie fields, is conclusive when considered in conjunction with the post-mortem findings.
10. Peracute cases are distinguished with difficulty from cases of poisoning whether of vegetable or mineral origin, and invariably a detailed investigation is necessary before a diagnosis can be ventured with any certainty. Anthrax is easily distinguished by microscopic examination of the blood.

Epizootology.

The incidence of the disease is confined to those areas where the environmental conditions favour the development of the tick. As has been mentioned previously *A. hebraeum* is distinctly fastidious in some of its requirements, and quite unwittingly full advantage was taken of this by the early settlers who knew that if Heartwater broke out, a move had merely to be made to a higher, colder, drier or even open area for the spread of the disease to cease immediately. This limitation of injection undoubtedly contributed to the belief that the disease was associated with the type of grazing and remained confined to certain plant successions.

The influence of climate does not end with its bearing upon the life cycle of ticks. Experience and observations over a number of years have indicated that Heartwater is a disease which varies in frequency of occurrence and severity with the season of the year. The early "trek farmers" realized this, and they had no compunction about moving their flocks of Merino sheep in the winter from the highveld areas, where the disease does not occur, to the Thorn country of Natal where the disease is rampant, but they knew that the return had to be commenced by September if heavy mortality from Heartwater was to be avoided. Cases do occur in the winter if the weather conditions are favourable for tick activity, but they are not common under farming conditions. In addition, there is evidence for the belief (Theiler, 1905, etc.) that cases which do occur during the winter are milder, with a lower mortality, than those encountered in the summer. The reason is quite obscure, but it would seem to stand in some relation to the influence of higher mean temperature on the development of the virus in the tick.

The fact that *A. hebraeum per se* is non-pathogenic, that the infection is not transmitted from one generation to another through the egg, and that the recovered animal does not remain a reservoir of

the virus, is sufficient explanation of the observation that Heartwater need not be a menace to progressive animal husbandry, even in those areas where the tick will thrive. This is clearly demonstrated over large areas in the Border Districts of the Eastern Province, the original home of the disease, where it was feared at one time that the breeding of Merino sheep would have to be abandoned as a commercial undertaking. Heartwater has been eradicated completely and with reasonable precautions (*cf. infra*) re-introduction and consequent heavy mortality need not be feared.

Treatment.

No drug or combination of drugs has been discovered which has any value as a specific in the treatment of Heartwater. At various times large numbers of official and unofficial preparations have been tried (Experiment S. 1563), but the results, when considered in the light of the great variability in mortality indicate that, though a few may have had some inconstant beneficial effect upon the course of the disease, none had any specific action comparable with the action of, say, trypan blue on *Piroplasma bigeminum*.

Treatment can be only symptomatic and even then is of little value when it is realized that complications are of rare occurrence, and the symptoms exhibited stand in direct relation to the pathological changes caused by the circulating noxa. Drenching, particularly in those cases showing marked nervous disturbances, is contra indicated, since there may be interference with deglutition which will make the production of an inhalation pneumonia a very real danger. Good hygiene, combined with rational feeding, is probably of the greatest importance.

From time to time the claims of calcium salts, both as a curative and a prophylactic, have been advanced. For instance, during the heavy mortality amongst stock after the introduction of the disease into the Eastern Province, Soga (1896) recommended lime and salt in the proportion of 1 : 2 as a prophylactic, and considered that three doses each of $\frac{1}{2}$ ounce, given at 8-day intervals, was sufficient to check the disease. This early expression of opinion, even though the grounds on which the recommendation was made are not given, becomes of considerable interest, since it has been found recently that in all probability calcium does play a role in the course of Heartwater, though the significance of that role is not clearly understood. As a preliminary to extensive work on the chemistry of the blood before, during and after a reaction, it was found by Malan in three sheep that there is an appreciable drop in the inorganic calcium content of the blood during the incubation period (from 10 to 8 mgm. per cent.) followed by a return to or slight rise above normal coincident with the initial rise in temperature, and a further fall during the course of the disease. Where this second drop was more pronounced (10-7.5 mgm. per cent.), the animal died; when the drop was not so pronounced (10-8.5 mgm. per cent.), the animals recovered. These results cannot be regarded as final, since confirmation by detailed examination of a large number of cases is necessary. Unfortunately this work has been unavoidably delayed. But, use of the observation has been made in an attempt to devise a satisfactory method of treatment which consists of the intravenous injection of 10 per

cent. calcium lactate (500 c.c.) to raise the inorganic calcium content of the blood immediately, the injection of magnesium hydroxide or magnesium citrate (5 gm.) 12 hours later, to maintain the high level of calcium and the simultaneous injecton of formalin (1-3 c.c. diluted 1 in 4) to modify the virus. The virus is not destroyed as was demonstrated by successful subinoculation into sheep at various times after the formalin injections, but in spite of non-specific viricidal action and in spite of somewhat conflicting results the author in collaboration with Pfaff is convinced that the treatment in cattle has had some beneficial effect upon the course of the disease and that the mortality amongst treated animals was appreciably lower than was anticipated from the known virulence of the strain of virus used. It would appear that this method of treatment yields the best results when commenced as soon as possible after the incidence of the fever reaction; this is difficult, since the rise in temperature often occurs at least three days before any diagnostic clinical symptoms become evident. In sheep completely negative results have been obtained. The method is being tested out under field conditions, but no data are as yet available. It is recommended tentatively that the formalin injections may be repeated on two successive days, but possibly the high calcium level of the blood is the imporatnt factor by preventing extensive oedemata and transudations of plasma. At any rate the observations open up a field of research into the chemistry of the blood and may be the basis of a rational method of treatment.

At this stage it is necessary to direct attention to the purgative action of formalin when given intravenously. This is marked in cattle where gastro-enteritis is constantly present, and frequently occurs in sheep but never when no lesions of enteritis are found at subsequent post-mortem.

Immune or hyperimmune serum has no value as a curative.

Immunity and Immunisation.

Immunity.

Field observations as well as immunity tests under laboratory conditions have shown that cattle, sheep and goats are capable of developing a definite immunity against Heartwater after recovery from a reaction. Since animals do develop an immunity under both natural and artificial conditions, the hope must always exist that, in spite of repeated failures in the past, there should be a possibility of developing some satisfactory method of preventive inoculation. Up to the present no such method has been evolved. It becomes necessary, therefore, to give in some detail the knowledge that has been accumulated of the nature, degree and development of immunity, and the methods of immunisation that have been tried.

It is a matter of great difficulty to build up an absolute immunity. As is the case with so many of the virus diseases, a conception of plurality of the virus strains must be accepted, and in the case of Heartwater the number of different strains appears to be legion. As a result of immunity and cross immunity tests conducted with a large

number of strains of virus collected from different parts of the Union from time to time, the following conclusions have been drawn:—

1. No immunity can be demonstrated in any animal (with the exception of those belonging to certain breeds which possess a degree of natural resistance) which has not passed through an attack of the disease. This applies in all cases whether immunisation is attempted by the injection of treated or untreated infective blood, activated or unactivated tick virus.

2. The severity of a Heartwater reaction does not appear to have any influence upon the degree of immunity, providing a definite reaction was produced.

3. An animal which has recovered from infection with one strain of virus usually possesses an immunity sufficient to protect it against subsequent infection with the same strain though that immunity is seldom so solid as completely to block out any further reaction. In one experiment (S. 4236) 34 sheep that had recovered from successful infection with a particular strain of virus, two died, one showed a very severe reaction but recovered, three a milder reaction and only two no evidence of febrile disturbance on being reinfected with the same strain.

4. A complete immunity is built up gradually by repeated reinfection. Two separate reactions to one strain produce a more solid immunity than one reaction. It is seldom possible to produce any deviation from normal at the third or fourth reinfection.

5. An animal which has recovered from infection with one strain of virus possesses a lower degree of immunity to a different strain than it does to the same strain. In one experiment (S. 4236) of 37 sheep, whose immunity was tested against an heterologous strain, five died, three showed very severe reactions but recovered, five less severe reactions and only one no sign of febrile disturbance.

6. Repeated reinfection with different strains of virus gradually builds up what appears to be a complete immunity against all strains. Up to the present no "master" strain as regards its immunising properties against all other strains has been isolated.

7. The virulence of a strain of virus, for which the percentage mortality it produces may be regarded as the index, has little influence on the degree of immunity it confers. For instance, one strain of virus which has known to produce a mortality of approximately 80 per cent., induced no greater degree of immunity against a mild strain from which the recoveries were more than 50 per cent., than against a third strain of exalted virulence. Moreover, animals which had recovered from the mild strain were found to possess a high degree of immunity against the virulent strains.

It is seen, therefore, that an absolute immunity is difficult to obtain and is only built up gradually by repeated infection. Recovery from one attack is usually sufficient to prevent a subsequent infection proving fatal, although a severe disease involving distinct economical loss, may be the result. This is borne out by observations under field conditions where animals have been observed to contract Heartwater on two or more occasions during the same year. Mortality, however, on heavily infected farms is chiefly confined to newly imported animals and young stock, the local adult animals having acquired an active immunity previously.

No conclusive data are available on the duration of immunity, but it appears most improbable that, if a resistance is once acquired, it is decreased during the life of the animal. Under field conditions any immunity is undoubtedly strengthened by reinfection. Spreull states that immunity persists for at least 18 months, i.e. through two seasons of potential infection. Recent experiments have shown that under laboratory conditions there is no decrease in immunity within twelve months.

The nature of the immunity is quite obscure, but it is gathered from the result of attempts at passive immunisation and serum treatment of clinical cases, that the antigenic properties are probably cellular and not humoral. This assertion is made in spite of the confirmed observation that the "parasitized" cell undergoes no reaction as a result of the presence of *Rickettsia ruminantium*.

Immunisation.

As has been mentioned above, numerous attempts have been made by different workers to elaborate a practical method of protective inoculation without any success. These may be enumerated as follows:—

1. In 1902 Dr. Purvis, a medical practitioner of Grahamstown, intimated that in his hands a particular method of immunisation had been attended by most satisfactory results. The method and technique he declined to reveal. The claims were tested by Spreull on forty "treated" sheep, to which were added twenty susceptible controls. The results as published by Spreull and Hutcheon (1905) were disappointing. No further progress has been reported since that time. Under the circumstances, it must be concluded that the Purvis method was of little or no value.

2. *Inoculation with Bile.*—In a manner analogous to that employed successfully in the immunisation of cattle against Rinderpest, Dixon (1898) conducted a number of experiments to ascertain whether the injection in varying amounts of bile alone, or bile simultaneously with the pericardial fluid obtained from typical cases of Heartwater, or bile simultaneously with virulent blood, would produce any immunity. He came to the conclusion that any antigenic properties of bile, if they exist, are negligible.

3. *Serum Virus Method.*—Immune cattle and sheep have been hyperimmunised by the intravenous injection of large quantities of blood repeated on three or more occasions, and attempts have been made to produce a potent anti-serum from horses in the same way, but

it was found that this anti-serum had no effect upon the course of the disease when injected before, together with, or after virulent blood. In those animals which recovered from the resulting reaction a definite immunity was established, but at no time was it possible to claim that the hyperimmune serum could be credited with any antigenic properties.

4. *Modified or Attenuated Blood Virus Method.*—Numerous attempts have been made to immunize animals by the intravenous or subcutaneous injection of virus modified by mechanical, chemical or thermal means. The methods employed have been:—

(*a*) Storage for 24 or 48 hours of either defibrinated or citrated blood in cold storage at ± 3° C., at room temperature, at 28° C. or at 37° C.

(*b*) Attenuation by the addition of 5 per cent. glycerine, 0.25 0.5 or 1 per cent. phenol.

(*c*) Citrated blood diluted in various proportions by the addition of normal saline.

(*d*) Blood heated at 50° C. for 10 minutes or 51° C. for seven minutes.

(*e*) Blood tapped from a donor before or after the reaction at various periods.

(*f*) Blood dessicated *in vacuo* over concentrated sulphuric acid or anhydrous calcium chloride at room temperature or at 37° C.

(*g*) Storage of macerated infective brain tissue at 3° C. in pure glycerine.

In each and every instance it was found that, if a reaction was initiated the recovered animal possessed the normal variable degree of immunity, but where there was no reaction no immunity was produced. In addition, the modified virus was either completely inert or it produced a reaction which differed in no respect from that set up by fresh virulent blood. Consequently, all the methods tried have had to be discarded as being too uncertain and far too dangerous for application to the field.

5. *Repeated Injections of Sublethal Doses of Virus.*—Here again the results proved that immunity was only established after a definite reaction which, however, could not be controlled within limits of safety.

6. *Repeated Subcutaneous Injection of Virus.*—This is a method which has been tried out extensively by Spreull, and was reported on favourably by him from time to time. In 1922 he advised the following doses which he considered safe:—

	First Vaccine.	Second Vaccine.	Third Vaccine.
Kids and Lambs	1.5 c.c.	1.5 c.c.	2.0 c.c.
Goats and Sheep (2-tooth) ...	3.0 c.c.	3.0 c.c.	4.0 c.c.
Goats and Sheep (4-tooth) ...	3.5 c.c.	3.5 c.c.	5.0 c.c.
Goats and Sheep (6-tooth to full mouth)	4.0 c.c.	4.0 c.c.	6.0 c.c.

The recommendation was that fresh citrated virulent blood, obtained from a reacting animal, was to be used for the vaccine and injections were to be made at intervals of not less than a month.

Apart entirely from the obvious difficulty of injecting accurately a small quantity of blood under field conditions, subsequent tests on similar lines have shown again that only those animals which react develop any immunity, and the reactions are uncertain and frequently dangerous.

Theiler and Du Toit attempted an extension of the method by giving the first injection subcutaneously and subsequent injections intravenously, with results that were no more encouraging. In addition, they made use of the simultaneous injection of large and small quantities of hyperimmune serum both subcutaneously and intravenously with identical results.

7. *Formalised Tissue Virus.*—Following the work of Laidlow and Dunkin on Distemper in the Dog, the author attempted to immunise Merino sheep by the use of formalised spleen, liver and brain tissue obtained from sheep at the height of the reaction. The identical technique was followed except that it was found impossible to standardize the infectivity of the material used as was suggested. Pooled material from several sheep reacting to strains of great virulence were used as an alterative. No reaction to the injection of the formalised product was observed in any sheep and, on immunity test, at varying intervals of time, it was found that no protection whatever had been conferred.

8. *Formalised Tick Virus.*—Prompted by the work of Spencer and Parker (1929) on Rocky Mountain Spotted Fever (subsequently similar work by Hindle on Yellow Fever appeared), several attempts at immunisation by the use of formalised tick virus were made. Larval *A. hebraeum* were fed on a reacting sheep during an Heartwater reaction. After moulting of the ticks the contained virus was activated by feeding to repletion on a bovine and were immediately ground up in a mortar and pestle with sterile sand and the addition of a small quantity of isotonic saline to which had been added 1.6 per cent. phenol and 0.4 per cent. formalin (40 per cent. formaldehyde). After thorough grinding the whole mass was transferred to a flask and an additional amount of saline-preservative added until the concentration was four ticks per c.c. After standing for 48 hours an equal quantity of sterile isotonic saline was added and the mixture stored for seven days at room temperature. The mixture was then diluted with a further equal quantity of saline, so that each c.c. now contained the equivalent of one tick. This method of gradual dilution ensures sterility of the final product and precipitation of the larger protein granules. Sheep were injected subcutaneously and intravenously with amounts varying from 1 to 10 c.c., but at an immunity test they proved to be fully susceptible. The results of this experiment were so poor as to suggest that no immunity can be produced by this method. It is felt, however, that as the virus in the tick may be stored before activation, and as it appears to be stable as regards virulence and infectivity after activation, it is the most reasonable basis for the continuation of immunological work. Modifications of the technique to include

accurate standardisation of the virus, and variations of formalin or other chemical concentration together with its subsequent neutralisation may prove solutions of the problem.

A similar attempt was made, using activated adult ticks instead of nymphae with no better results, but again it is felt that modifications of technique may prove successful.

Prophylaxis.

Various drugs have been recommended from time to time as prophylactics, e.g. Hutcheon (1898) recommended one tablespoonful of a mixture of ol. terebinth 1 gallon, ol. lini 1 gallon, Ferri sulph. 2 lb. and sulphur 2 lb. to be given to sheep every 2-3 months; Nel recommended alum; several writers recommended the use of salt and lime licks, but it is certain that none of these measures are of any appreciable value in practice.

With our present knowledge the only rational prophylactic of any importance is the elimination of infected ticks. At first sight it would appear that this is a difficult matter since the infection is not lost by ticks feeding on an insusceptible host, and is not always lost after feeding on a suspectible host, but the problem is simplified considerably by the inability of the virus to persist through the egg from one generation of tick to another. It must be borne in mind also that the tick itself is non-pathogenic, and only becomes a menace after it has fed on an animal during or shortly after an attack of the disease. Consequently, when once the virus has been eliminated no further cases need be encountered in spite of the presence of *A. hebraeum*, unless an animal containing the virus in the blood is imported on to the farm to re-establish infection in the tick population.

It is not within the scope of this article to deal with the problem of tick eradication, but it must be stated that systematic dipping of all animals in fluid of correct strength is the only satisfactory method of reducing the degree of tick infestation at present known. With regard to the genus *Amblyomma* several points must be taken into consideration:—

1. All stages of the tick are capable of feeding to repletion in as short a period as four days, consequently allowing one day for the arsenic retained on the skin of the animal to prevent reinfestation, dipping should be carried out at intervals of not less than five days.
2. The proboscis of the tick is fairly long and this fact in conjunction with the known resistance to destruction by arsenical dips, complicates the problem.
3. The tick is an ubiquitous feeder so that the regular dipping of all domestic animals becomes a necessity.
4. The minimum arsenical concentration necessary to kill the tick is not definitely known. Considerable additional research work is necessary to throw light on this point as well as to determine the exact mechanism of destruction by arsenical dips.

In practice it has been found that 5-7 day dipping in a fluid containing 2 lb. of sodium arsenite per 100 gallons gives excellent results. Dipping at 14 day intervals in a stronger fluid is less successful, and dipping at 3 day intervals in a weaker solution is not so effective in killing the tick besides having a harmful effect on the animal when enforced over long periods of time.

The measures to be adopted to prevent the incidence of the disease amongst both large and small stock may be considered under several headings:—

1. *To Prevent the Introduction on to a Clean Farm.*—In localities outside the natural habitat of the Bont tick, e.g. the Highveld, importations of all classes of stock from any area may be made with impunity.

In the low veld and the known Heartwater areas, importations should be regarded with disfavour, except from areas where the disease does not occur, e.g. the Karroo. Otherwise animals should only be admitted after passage through a series of two or three quarantine camps, in each of which they should remain for a period of twenty-five days. Remaining for 25 days in the first camp will ensure the dropping of all *Amblyomma* from their hosts, and the animals will have been removed before the completion of any moulting. Retention for twenty-five days in the second camp will ensure that all animals in the incubation stages of the disease are detected, and will prevent the dropping of any infected ticks from such animals outside the quarantine camps. Isolation in a third camp will only become necessary should any cases of Heartwater occur in camp 2. These camps must thereafter be treated as described for infected farms.

In spite of every care being taken with the importation of stock, it is a matter of difficulty to prevent the virus gaining entrance, since the movement of game and small wild animals, e.g. hares, cannot be controlled. Such animals are capable of carrying infected nymphae considerable distances. The danger which they afford can only be minimised by the active co-operation of all the farmers in the area, in an attempt to reduce the total number of ticks by systematic dipping of all domestic animals and to reduce the number of infective ticks by strict isolation of all Heartwater cases as soon as any suspicious clinical symptoms become apparent, so that ticks which have fed on the virus reservoir may be concentrated in one spot to facilitate destruction.

2. *To Prevent the Spread amongst a Herd or Flock.*—The only method which can be relied upon to give immediate results, is the removal of all infected animals from the area to a locality where conditions are not favourable for the development of the tick. The disease will naturally have to take its course in all animals which have picked up the infection already, but there is no chance of direct infection and no futher cases will develop. The long incubation period of some cases must be borne in mind so that symptoms may make their appearance up to a month after departure from the infected farm.

If it is not possible to move the animals the only alternative is to limit the possibility of infection by restricting the grazing to the relatively higher and more open portion of the farm, and to enforce

regular 5-day dipping. Immune or susceptible animals should be run on the lower lying and more sheltered portions as a means of collecting and transporting the ticks to the dip for destruction.

3. *To Eradicate the Disease on an Infected Farm.*—This can be done only by withdrawing all susceptible animals which are capable of acting as reservoirs for the infection of ticks for two summer seasons or a total period of at least 20 months. During that time all infected ticks will either have completed their entire life cycle and so have cleared themselves of infection or they will have succumbed to starvation. In the meantime stocking the farm with insusceptible animals, e.g. horses or donkeys, which can be dipped regularly, is an excellent procedure in order to reduce the total tick population. In practice it has been found possible to eliminate danger of heavy mortality by introducing in the first instance local immune animals or Persian, or cross-bred Persian sheep which are capable of standing prolonged 7-day dipping and only later to introduce Merino sheep. By this method wool growing and the farming of improved breeds of cattle have been made profitable ventures two years after commencement of the campaign.

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APPENDIX.

In this appendix details are given of experiments upon which the subject-matter of the foregoing article has been based in fact. Where possible the name of the officer in charge of the experiment is given as a basis of due acknowledgment.

Filtration of the Virus.

Experiment S. 544 *of* 12.9.19. D. T. Mitchell.

Object: To ascertain if the virus of Heartwater is a filter passer.

Method: Two sheep injected intraj. with 10 c.c. serum from a reacting sheep after passing through a Berkefeld W candle with 30 c.c. of normal saline. Injections made 3½ hours after bleeding. As controls, two sheep with unfiltered blood at the same time.

Result: Control sheep reacted severely but recovered. Other two sheep showed a very slight thermal disturbance but no clinical symptoms.

Experiment S. 597 *of* 20.10.19. D. T. Mitchell.

Object: To control Ex. S. 544 in ascertaining if the virus of Heartwater is a filter passer.

Method: As Ex. S. 544 above.

Result: Both sheep again showed a slight febrile disturbance, while of the controls both reacted and one died.

Experiment S. 617 *of* 26.11.19. D. T. Mitchell.

Object: To control Ex. S. 597 in ascertaining if the virus of Heartwater is a filter passer.

Method: As Ex. S. 597, but blood diluted 1:5 before filtration.

Result: Both sheep showed a very slight thermal reaction with no clinical symptoms, while both control sheep reacted severely but recovered.

Experiment S. 2987 *of* 22.2.27. H Graf. *Cf.* Longevity of the virus.

Experiment S. 4179 *of* 22.2.27. R. A. Alexander.

Object: To ascertain if the virus of Heartwater is filterable.

Method: Virulent blood was centrifuged to remove serum. To the corpuscles 10 times normal saline was added in an amount equal to one-tenth of the volume

of removed saline. After standing one hour 9 times this volume of distilled water was added and allowed to stand for one hour. This haemolysed material was then filtered—

(*a*) through a thin layer of paper pulp by aspiration;

(*b*) through a Seitz filter, whose porosity was controlled by failure to pass *B. prodigiosus*.

Whole citrated blood served as control. All injections made at same time 5 hours after tapping blood from the reacting donor.

Result: Through paper pulp—

15 c.c.—Severe reaction.
10 c.c.—Severe reaction.
1 c.c.—Negative.

Through Seitz filter—

15 c.c.—Negative.
10 c.c.—Negative.
1 c.c.—Negative.

Whole citrated blood—

5 c.c.—Severe reaction.
0.5 c.c.—Negative.

Localisation and Concentration of the Virus in the Blood.

Experiment S. 424 *of* 20.5.19. D. T. Mitchell.

Object: To ascertain if the virus is present in fresh blood serum.

Method: Blood collected in citrate from a reacting sheep. Plasma removed by centrifuging.

Result: One sheep 10 c.c. plasma intrajugularly—slight thermal reaction; no clinical symptoms.

1 sheep 40 c.c. plasma intrajug.—No reaction.
1 sheep 40 c.c. plasma intrajug.—No reaction.
2 sheep 10 c.c. whole blood—Both reacted and 1 died.

These results are not satisfactory, as sheep were injected 24 hours after bleeding, while controls were injected at time of bleeding.

Expriment 440 *of* 27.5.19. D. T. Mitchell.

Object: To ascertain if Heartwater can be produced by the injection of the fluid obtained from the plural cavity of a sheep which died of Heartwater.

Result: 1 sheep 20 c.c. of fluid subcut.—No reaction.
1 sheep 20 c.c. of fluid intraj.—No reaction.

No control was included.

Experiment S. 448 *of* 2.6.19. D. T. Mitchell.

Object: To ascertain whether washed corpuscles, or serum prepared from known infected material can produce the disease in susceptible animals.

Result: 2 sheep injected intraj. 10 c.c. washed corpuscles—both died.
3 sheep injected intraj. 10, 40, 50 c.c. plasma—all died.
2 sheep injected intraj. 10 c.c. citrated blood—both died.

Inoculations made $1\frac{3}{4}$ hours after bleeding.

Experiment 465 *of* 18.6.19. D. T. Mitchell.

Object: To ascertain if Heartwater can be produced by the injection of the fluid obtained from the plural cavity of sheep which died of Heartwater.

Method: 2 sheep injected intrajugularly with 10 c.c. of fluid in plural cavity of a reacting sheep.

Result: A definite but very mild reaction assured in both sheep.

Experiments No. 3219 *of* 23.9.27. E. M. Robinson.

Object: To see if the virus can be centrifugalised.

Method: Virulent blood centrifugalised, serum removed and corpuscles washed in saline immediately prior to injection into susceptible sheep.

Result: 1 sheep 5 c.c. plasma intrajugularly—No reaction.
1 sheep 5 c.c. corpuscles after one washing intraj.—No reaction.
1 sheep 5 c.c. corpuscles after two washings intraj.—No reaction.

No controls were included in the experiment .

Experiment S. 4229 *of* 22.7.30. R. A. Alexander.

Object: To determine the localisation of the virus in the blood of a reacting sheep.

Method: Blood from a reacting sheep was tapped into citrate. Plasma separated by rapid prolonged centrifuging. Corpuscles washed three times in normal saline and then saline added to bring count back to that of the original blood. White corpuscles removed by removing the entire buffy coat after centrifuging, and by filtration through a 20 c.cm. tube plugged with non-absorbent cotton wool. Seitz filtrate obtained by filtering washed corpuscles. Sheep bled 9 a.m. All injections 2-2.30 p.m. of same day.

Result:

D.O.B. No. of Sheep.	Material Injected.	Amount Injected in c.c.	Reaction.	Result.
28876	Whole blood.....	10·0	Typical.....	Died (Heartwater).
28461	,, ,, ...	10·0	Typical.....	Died (Heartwater).
28520	,, ,, ...	1·0	Marked.....	Recovered.
28791	,, ,, ...	1·0	Typical.....	Recovered.
28511	,, ,, ...	0·1	Mild.....	Recovered.
28915	,, ,, ...	0·1	Mild.....	Recovered.
28895	,, ,, ...	0·01	No reaction....	—
28999	,, ,, ...	0·01	Mild.....	Recovered.
28846	Red and white corpuscles	10·0	Typical.....	Recovered.
22992	,, ,, ,,	10·0	Typical.....	Died.
28866	,, ,, ,,	1·0	Mild.....	Recovered.
28881	,, ,, ,,	1·0	Mild.....	Recovered.
23271	,, ,, ,,	0·1	No reaction....	—
28700	,, ,, ,,	0·1	No reaction....	—
28666	,, ,, ,,	0·01	No reaction....	—
28954	,, ,, ,,	0·01	No reaction....	—
28630	Red corpuscles alone (by centrifuge)	10·0	Typical..	Killed *in extremis*.
28658	,, ,, ,,	1·0	Mild.....	Recovered.
28871	,, ,, ,,	1·0	Mild.....	Recovered.
28735	,, ,, ,,	0·1	Mild.....	Recovered.
28824	,, ,, ,,	0·1	No reaction....	—
23642	,, ,, ,,	0·01	No reaction..	—
28902	,, ,, ,,	0·01	Mild.....	Recovered.
28994	Serum alone.....	10·0	No reaction....	—
28870	,, ...	10·0	No ,,	—
28935	,, ...	1·0	No ,,	—
28930	,, ...	1·0	No ,,	—
24564	,, ...	0·1	No ,,	—
24309	,, ...	0·1	No ,,	—
28882	,, ...	0·01	No ,,	—
24782	,, ...	0·01	No ,,	—
28802	Red corpuscles alone (Hornby's technique)	10·0	Typical.....	Died.
28484	,, ,, ,,	10·0	Typical.....	Recovered.
28919	,, ,, ,,	1·0	Typical.....	Recovered.
28894	,, ,, ,,	1·0	Typical.....	Recovered.
28676	,, ,, ,,	0·1	No reaction....	—
28847	,, ,, ,,	0·1	No ....,,.....	—
28795	,, ,, ,,	0·01	No ....,,.....	—
28808	,, ,, ,,	0·01	No ....,,.....	—
28572	Seitz filtrate.....	10·0	Mild.....	Recovered.

Experiment S. 3860 *of* 10.4.29. R. A. Alexander.

Object: To ascertain the localisation of the virus of Heartwater.

Method: Sheep 21130 bled during a reaction. The blood was defibrinated by gentle shaking with beads. After centrifuging the separate corpuscles and serum the corpuscles were washed three times in isotonic saline and the serum centrifuged for 30 minutes at 400 revolutions per minute to separate out all corpuscles. Saline was added to the corpuscles to bring back the count to that of the blood, and thick smears were made of the serum to exclude the presence of corpuscles.

Result: 10 c.c. serum: Sheep 21167—No reaction. On immunity test reacted and recovered.
Sheep 22124—No reaction. On immunity test reacted and recovered.

5 c.c. corpus: Sheep 20706—Mild reaction. On immunity test no reaction.
Sheep 22105—No reaction. On immunity test reacted and recovered.

Control 5 c.c. defibrinated blood. Good reaction. No reaction.

Longevity of the Virus.

Experiment S. 241 *of* 11.12.18. D. T. Mitchell.

Object: To test the longevity of the Hearwtater virus in vitro.

Method: After storage at 3° C. for 24, 48, and 72 hours each of the following were injected into two susceptible sheep in 5 c.c. amounts intrajugularly. Defibrinated blood, blood in O.C.G. mixture, and serum.

Result: 1. Heartwater transmitted only by inoculation of defibrinated blood and not by O.C.G. blood or serum at 24th hour.

2. Virus active to slight extent in defibrinated blood, but not active in O.C.G. blood or serum at 48th hour. The two sheep that received defibrinated blood gave a slight reaction but proved susceptible on immunity test.

3. Defibrinated blood, O.C.G. mixture and serum all inactive at 72nd hour.

Note.—All non-reactors proved fully susceptible on immunity test.

Experiment S. 2952 *of* 1.2.27. P. J. du Toit.

Object: To test for virulency blood that has been—

(*a*) defibrinated
(*b*) oxalated

and kept at room temperature for 12, 24, 36, and 48 hours. Each sheep receiving 10 c.c. intrajugularly.

Result: 1. Defibrinated blood kept for 12 hours produced Heartwater, the reaction being proved by subinoculation. After 12 hours it produced no reaction.

2. Oxalated blood was not virulent at any time (probably due to the 5 per cent. glycerine contained in the mixture).

Experiment S. 2953 *of* 1.2.27. P. J. du Toit.

Object: To note the virulence of the virus in a mixture of 5 per cent. and 50 per cent. glycerine.

Method: Blood from a reacting sheep bled into oxalate + 5 per cent. and 50 per cent. glycerine and injected into susceptible sheep after standing at room temperature for 9 hours.

Result: None of the sheep reacted. Control sheep died of Heartwater.

Experiment S. 2984 *of* 21.2.27. H. Graf.

Object: To study the resistance of Heartwater virus.

Method: Susceptible sheep are injected intrajugularly with 5 c.c. of defibrinated and citrated blood and O.C.G. blood fresh and 8 hours old.

Result: 5 c.c. defibrinated blood fresh: Reaction.
5 c.c. defibrinated blood 8 hours old: Reaction.
5 c.c. fresh citrated blood: Reaction.
5 c.c. citrated blood (8 hours old): Reaction.
5 c.c. O.C.G. blood fresh: No reaction.
5 c.c. fresh undefibrinated blood: Reaction. Died.
5 c.c. blood 8 hours old: Reaction.

Experiment S. 2987 *of* 22.2.27. H. Graf.

Object: To study the resistance of Heartwater virus and filterability through a Seitz filter.

Method: Sheep injected as indicated with material obtained from a reacting sheep.

Result: 5 c.c. intrajugularly fresh oxalated blood: Reaction.
5 c.c. oxalated plasma (centrifuged): Reaction.
5 c.c. oxalated corpuscles (centrifuged): Reaction. Died.
5 c.c. oxalated blood (12 hours old): No reaction.
20 c.c. oxalated filtered plasma (diluted 1:4): No reaction.
20 c.c. haemolysed filtered corpuscles (diluted 1:4): No reaction.
20 c.c. haemolysed corpuscles (1:4) unfiltered: No reaction.

Experiment S. 3011 *of* 21.3.27. H. Graf.

Object: To observe whether 5 or 50 per cent. glycerine concentration will interfere with the viability of the virus.

Method: Defibrinated virulent blood is mixed with glycerine and injected intrajugularly into susceptible sheep.

Result: 10 c.c. defibrinated blood + 5 per cent. glycerine injected on day of bleeding: No reaction.
10 c.c. defibrinated blood + 50 per cent. glycerine injected on day of bleeding: No reaction.
5 c.c. defibrinated blood + 5 per cent. glycerine injected after 14 days: No reaction.
5 c.c. defibrinated blood+50 per cent. glycerine injected after 14 days: No reaction.
2 control sheep 5 c.c. fresh defibrinated blood: Reaction.

Experiment S. 3443 *of* 20.3.28. E. M. Robinson.

Object: To see how long the virus of Heartwater will live in defibrinated blood + 1 per cent. glucose.

Method: Susceptible sheep injected with 5 c.c. intrajugularly of the mixture after 24, 48, and 72 hours.

Result: 1. After 24 hours the sheep reacted and proved immune.
2. After 48 hours the sheep did not react, and later proved susceptible to Heartwater.
3. After 72 hours the sheep did not react, and later proved susceptible to Heartwater.
4. Control sheep reacted and later proved immune.

Experiment S. 4237 *of* 12.8.30. R. A. Alexander.

Object: To ascertain the longevity of the virus in an emulsion of activated ticks and the immunity produced by the intravenous injection of such emulsion.

Method: *A. hebraeum nymphae* batch 1138 Ca produced typical Heartwater in C. 3541, the ticks having been injected by feeding on 2892 during a reaction. The reaction in C. 3541 was confirmed by subinoculation into Sheep 28984 which reacted severely but recovered. The ticks on engorgement were emulsified by grinding with sand in saline and injected into susceptible sheep at intervals of 24 hours, each sheep receiving the equivalent of 1 tick.

Result:

Sheep.	Date of Injection.	Age of Emulsion.	Result.	Immunity Test.
28787.....	23/8/30	1 day..	Negative.....	Severe reaction. Recovered.
27549.....			Slight fever.... 11–16	,, ,, ,,
24309.....	25/8/30	3 days.	Negative.....	,, ,, ,,
28930.....			,, ...	,, ,, ,,
28824.....	26/8/30	4 days.	,, ...	Died Heartwater.
28808.....			,, ...	Severe reaction. Recovered.
28870.....	27/8/30	5 days.	,, ...	,, ,, ,,
28985.....			,, ...	Died Heartwater.
28795.....	28/8/30	6 days.	,, ...	Severe reaction. Recovered.
28935.....			,, ...	,, ,, ,,
28828.....	29/8/30	7 days.	,, ...	,, ,, ,,
26655.....			,, ...	Died Heartwater.
27398.....	30/8/30	8 days.	,, ...	Severe reaction. Recovered.
27422.....			,, ...	Died Heartwater.

Of the controls in immunity test 2 reacted and recovered and 1 died out of 3.

Preservation of the Virus.

Experiment S. 3097 *of* 11.6.27. E. M. Robinson.

Object: To attempt to preserve the virus of Heartwater.

Method: Blood taken from cases of Heartwater during the first days of the reaction, defibrinated by gentle stirring with a glass rod and injected intrajugularly into susceptible sheep after keeping for 7 days.

Result: 2 c.c. defibrinated blood + liquid paraffin kept at room temperature: No reaction.
2 c.c. defibrinated blood + 2 per cent. formalin kept at 0° C.: No reaction.
2 c.c. defibrinated blood + isotonic sodium citrate at room temperature: No reaction.
2 c.c. defibrinated blood + isotonic Locke gelatine dextrose at room temperature: No reaction.
2 c.c. defibrinated blood + isotonic dextrose kept at room temperature: No reaction.
2 c.c. defibrinated blood kept at room temperature: No reaction.

Note.—All non-reactors proved susceptible and reacting controls immune on immunity test.

Experiment S. 3147 *of* 29.7.27. E. M. Robinson.

Object: To attempt the preservation of the virus of Heartwater in the blood.

Method: Blood was tapped from a reacting sheep at the height of the reaction and defibrinated by stirring gently with a rod without any beads. After addition of the materials indicated when kept for 7 days at 3° C. both aerobically and anaerobically susceptible sheep were injected intrajugularly.

Result: 5 c.c. defibrinated blood + isotonic dextrose aerobically for 7 days at 3°: No reaction.

5 c.c. defibrinated blood + isotonic dextrose anaerobically for 7 days at 3°: No reaction.

5 c.c. defibrinated blood + gelatine Locke solution aerobically for 7 days at 3°: No reaction.

5 c.c. defibrinated blood+gelatine Locke solution anaerobically for 7 days at 3°: No reaction.

5 c.c. defibrinated blood fresh: Reaction.

NOTE.—Non-reactors proved susceptible on immunity test.

Experiment S. 3219 *of* 23.9.27. E. M. Robinson.

Object: To attempt the preservation of the virus in Heartwater blood.

Method: As S. 3147 above.

Result: 5 c.c. defibrinated blood + glucose 1 in 100 after 7 days at 28° C.: No reaction.

5 c.c. defibrinated blood + glucose 1 in 50 after 7 days at 28° C.: No reaction.

NOTE.—Non-reactors proved susceptible on immunity test.

Experiment S. 3229 *of* 1.10.27. E. M. Robinson.

Object: To attempt the preservation of virus as contained in Heartwater blood.

Method: As S. 3219.

Result: 5 c.c. defibrinated blood + 1 c.c. 2.8 per cent. saccharose after 8 days at 28° C.: No reaction.

5 c.c. defibrinated blood + 2 c.c. 2.8 per cent. saccharose after 8 days at 28° C.: No reaction.

5 c.c. defibrinated blood + 5 c.c. 2.8 per cent. saccharose after 8 days at 28° C.: No reaction.

Experiment S. 3881 *of* 24.4.29. R. Alexander. *Cf.* Attenuation of the virus.

ATTENUATION OF THE VIRUS.

Experiment S. 2110 *of* 21.4.25. A. Theiler.

Object: To determine whether strain of Heartwater virus C. 860 will prove to be an attenuated one.

Method: Passage by subinoculation into susceptible sheep at the height of each successive reaction. Injections made in 5 c.c. amounts intrajugularly.

Result: In 21 generations the virulence had not decreased but had apparently increased.

Experiment S. 211 *of* 21.4.25. A. Theiler.

Object: To determine whether strain of Heartwater C. 853 will prove to be an attenuated one.

Method: As S. 2110.

Result: In 9 generations using 22 sheep and 3 goats the virulence of the strain appeared to increase in the later generations.

Experiment S. 2112 *of* 21.4.25. A. Theiler.

Object: To determine whether strain of virus C. 1024 will prove to be attenuated.

Method: As S 2110.

Result: In 6 generations 13 sheep and 3 goats were injected, of which 8 sheep and 3 goats died, the virulence of the strain increasing in the higher generations.

Experiment S. 2113 *of* 21.4.25. A. Theiler.

Object: To determine whether the Krugersdorp strain of Heartwater will prove to be attenuated.

Method: As S. 2110.

Result: In 9 generations 18 sheep, 3 goats, and 1 bovine were injected. Of these, 7 sheep, 2 goats, and 1 bovine died, all the deaths occurring in the higher generations. The virulence therefore appears to have increased.

Experiment S. 3881 *of* 24.4.29. R. A. Alexander.

Object: To attenuate or retain the virulence of the virus by desiccation in vitro.

Method: (A) 10 c.c. blood was tapped from Sheep 19319 at the height of the reaction and placed in a desiccator over concentrated sulphuric acid at a pressure of 10 mm. of Mercury. Desiccation was complete at the end of 48 hours. Residue after storage for 9 days was dissolved in 20 c.c. of isotonic saline and injected into 2 sheep immediately.

(B) Blood from reacting Sheep 19966 was dried in vacuo in a Shärers apparatus at 37° C. over calcium chloride (anhydrous). Desiccation was complete in 24 hours, when the residue was dissolved in saline after 24 hours, 4 days, and 8 days, and injected in 10 c.c. amounts into sheep.

Result: (A) 19319—No reaction: Immunity test severe reaction.
20033—No reaction: Died traumatic peritonitis.
(B) 24 hours: 22516: No reaction.
22875: No reaction.
4 days: 22646: No reaction.
23060: No reaction.
8 days: 22998: No reaction.
23042: No reaction.

On immunity test all sheep reacted.

The Behaviour of the Virus in the Recovered Animal.

Experiment S. 1868 *of* 31.10.24. P. J. du Toit.

Object: To determine whether cattle that have recently passed through Heartwater will act as reservoirs of the virus.

Method: Calf 928 infected on ears with *A. hebraeum* batch 517 of 12.8.24. Tests made by intrajugular injection of 10 c.c. of blood into susceptible sheep.

Result: 31.10.24: 2 sheep both recovered after reaction.
14.11.24: 2 sheep both reacted and died.
27.11.24: 2 sheep both reaeted and 1 died.
10.12.24: 2 sheep both reacted and recovered.
24.12.24: 2 sheep. No reactions.
10.1.25: 1 sheep. Very mild thermal reaction with no clinical symptoms.
26.1.25: 6 sheep. 2 very mild reaction, 4 sheep no reaction.

Experiment S. 1938 *of* 3.12.24. P. J. du Toit.

Object: To determine whether sheep previously injected with two strains of Heartwater retain the infeetion in their blood and for how long.

Method: Five sheep had recovered from infection with two strains of virus. The period of incubation was 6 days and the temperature had returned to normal on the 21st day. Injections into susceptible sheep were then made.

Result: On 21st day the virus was still present in that blood, but it had disappeared by the 36th day after infection.

Experiment S. 2429 *of* 24.2.26. P. J. du Toit.

Object: To test for viruleney at 14-day intervals the blood of sheep 13952 and 13833 that in experiment S. 2278 had passed through Heartwater.

Method: At 14-day intervals inject susceptible sheep with 5 c.c. fresh blood intrajugularly.

Result: Three weeks after the reaction no reaction was produced.

Experiment S. 2544 *of* 5.5.26. P. J. du Toit.

Object: To test for virulence (virus reservoir) blood of sheep 12207 that showed an unusually long and abnormal reaction taken at the conclusion of the reaction and again 12 days later.

Method: Sheep 12207 injected 6.11.26 reacted from 18.4.26-4.5.26. Susceptible sheep were injected with 5 c.c. fresh blood intrajugularly on the dates mentioned.

Result: On 5.5.26: Both sheep reacted and one died.
17.5.26: One sheep reacted (only one sheep injected).
27.5.26: One sheep injected. No reaction.

Experiment S. 2845 *of* 26.11.26. P. J. du Toit.

Object: To ascertain how long the blood of an ox will remain infective after the temperature has returned to normal after a reaction to Heartwater produced by the intravenous injection of virulent blood.

Method: The ox received 5 c.c. virulent blood intrajugularly on 26.11.26. The Heartwater reaction commenced on 8.12.26 and temperature had returned to normal on 16.12.26. Subinoculations were then made into susceptible sheep as indicated.

Result: 22.12.26: 2 sheep injected. Both reacted.
3.1.26: 2 sheep injected. Both reacted.
14.1.26: 2 sheep injected. No reaction.
24.1.26: 2 sheep injected. No reaction.

Experiment S. 2875 *of* 22.12.26. P. J. du Toit.

Object: To determine how long blood of cattle recovered from Heartwater will remain infective.

Method: Ox 1671 showed Heartwater reaction from 8.12.26-15.12.26. From time to time, as indicated, blood was injected into susceptible sheep in 5 c.c. amounts intrajugularly.

Result: 7 days after reaction—2 sheep: Both reacted and both recovered.
19 ,, ,, 2 sheep: Both reacted and one died.*
30 ,, ,, 2 sheep: No reaction.
35 ,, ,, 2 sheep: One very mild reaction,† one no reaction.
41 ,, ,, 2 sheep: One reaction†, one no reaction.
54 ,, ,, 2 sheep: No reaction.
66 ,, ,, 2 sheep: No reaction.
85 ,, ,, 2 sheep: No reaction.

Non-reactors on immunity test all reacted.

* Reaction proved to be Heartwater by subinoculation into 2 sheep, each of which reacted.

† Reaction proved to be Heartwater by subinoculation into two sheep, each of which reacted.

Experiment 2936 *of* 28.1.27. P. J. du Toit.

Object: To ascertain how long the blood of an animal remains infective after recovery from an attack of Heartwater.

Method Ox 1757 was injected with 5 c.c. virulent blood intrajugularly on 12.1.27; commenced Heartwater reaction on 26.1.27 and temperature returned to normal on 7.2.27, but a slight febrile remission occurred from 8.2.27-14.2.27. From time to time as indicated susceptible sheep were injected with 5 c.c. intrajugularly.

Result 7.2.27—2 sheep: Both reacted and both died.
14.4.27—2 cattle: 1 gave slight thermal reaction* and 1 no reaction.
21.2.27—2 sheep: No reaction.
3.3.27—2 sheep: No reaction.
12.3.27—2 sheep: No reaction.

Non-reactors proved susceptible on immunity test.

* Proved Heartwater reaction by injection into 2 sheep, both of which reacted to Heartwater.

Experiment 3079 *of* 17.5.27. P. J. du Toit.

Object: To test the infectivity of ticks fed on Ox 1757 in Ex. S. 2909 during and at various times after a Heartwater reaction.

Method: Amblyomma hebraeum larvae batch 892, 893, 895, 897 were used to transmit infection (as vectors to the nymphal stage). Ox 1757 (donor) reacted from 26.1.27-7.2.27.

Result:

Fed as larvae.	*Moulted.*	*Fed as nymphae on*	*Result.*
28.1.27- 2.2.27	2.3.27	Goat 15722	Reacted and recovered.
4.2.27- 9.2.27	9.3.27	Goat 16199	Reacted and died.
14.2.27-19.2.27	25.3.27	Goat 16209	Reacted and died.
21.2.27-25.2.27	25.3.27	Goat 16221	Reacted and died.

Experiment S. 4020 *of* 30.9.29. *Cf.* Duration of immunity.

MULTIPLICATION OF THE VIRUS IN ANIMALS OTHER THAN OVINES AND BOVINES.

Experiment S. 259 *of* 20.12.18. D. T. Mitchell.

Object: (*a*) To ascertain the susceptibility to Heartwater of:—

1. Cattle.
2. Sheep.
3. Goats.
4. Dogs.
5. Small laboratory animals.

(*b*) To ascertain whether other animals harbour the virus.

Method: Inject virulent blood as indicated and where a reaction occurs subinoculate into susceptible sheep.

Result: Cattle—10 c.c. defibrinated blood intrajugularly: Negative.
Sheep—10 c.c. defibrinated blood intrajugularly: Reacted, died.
Goats—10 c.c. defibrinated blood intrajugularly: Reacted, died.
Dogs—10 c.c. defibrinated blood intrajugularly: Negative.
Rabbits—10 c.c. defibrinated blood intraperitoneally: Negative.
Guinea-pigs—10 c.c. defibrinated blood intraperitoneally: Negative.
Rats—10 c.c. defibrinated blood intraperitoneally: Negative.
Mice—3 c.c. defibrinated blood intraperitoneally: Negative.

Experiment S. 398 *of* 7.5.19. D. T. Mitchell.

Object: To ascertain what animals other than sheep and goats are susceptible to Heartwater.

Result: Dogs (2)—20 c.c. intrajugularly of mixture of blood of 2 reacting sheep: Negative.
Horses (3)—200 c.c. intrajugularly of mixture of blood of 2 reacting sheep: Negative.
Cattle (2)—100 c.c. intrajugularly of mixture of blood of 2 reacting sheep: Negative.

Experiment S. 410 *of* 13.5.19. D. T. Mitchell.

Object: To ascertain if multiplication of the virus occurs in insusceptible animals.

Method: Dogs were subinoculated with 20 c.c. of blood intrajugularly as follows:—

Result:

Date of Injection.	Injected From.	Injected Into.	Injected From.	Injected Into.
7/5/19	Reacting sheep.	Dog 1545, no reaction	Reacting sheep...	Dog 1546, no reaction.
13/5/19	Dog 1545.....	Dog 1527, slight fever	Dog 1546.....	Dog 1524, no reaction.
19/5/19	Dog 1527.....	Dog 1496, fever..	Dog 1524.....	Dog 1482, no reaction.
25/5/19	Dog 1496.....	Dog 1431, fever...	Dog 1482.....	Dog 1475, no reaction.
31/5/19	Dog 1431.....	Dog 1497, no reaction.	Dog 1475.....	Dog 1504, fever.
6/6/19	Dog 1497.....	Dog 1481, no reaction	Dog 1504.....	Dog 1483, no reaction.
12/6/19	Dog 1481.....	Dog 1540, slight fever	Dog 1483.....	Dog 1499, fever.
18/6/19	Dog 1540.....	Dog 1492, fever..	Dog 1499.....	Dog 1494, fever.
24/6/19	Dog 1492.....	Dog 1486, fever..	Dog 1494.....	Dog 1488, fever.
28/6/19	Dog 1492.....	Sheep 15665, no reaction	Dog 1492.....	Sheep 13855, fever.

NOTE.—No blood smear examinations of the dogs were made. Fever reactions in the dogs occurred from 4th-9th day. In sheep from 13th day.

Experiment S. 1564 *of* 15.1.24. P. J. du Toit.

Object: To infect guinea-pigs and rabbits with Heartwater.

Method: 1 c.c. of fresh defibrinated blood injected subcutaneously into 2 rabbits, 4 guinea-pigs, and then subinoculated into 2 goats and 2 guinea-pigs from one guinea-pig.

Result: No reaction in any of the animals.

Experiment S. 3150 *of* 1.8.27. E. M. Robinson.

Object: To see whether the blood of a rat which gave a temperature reaction after inoculation with Heartwater blood contains the virus.

Method: 5 c.c. of fresh blood of the rat injected intravenously into one sheep.

Result: No reaction.

Experiment S. 3991 *of* 5.9.29. R. A. Alexander.

Cf. Active Immunisation using tick virus.

Experiment S. 4225 *of* 17.7.30. R. A. Alexander.

Object: To ascertain whether the virus of Heartwater will either retain its infectivity or multiply in equines.

Method: Intravenous subinoculation from a reacting bovine into two horses and then progressively into other horses on the 8th day in 10 c.c. amounts.

Result: In four generations no reactions due to Heartwater reactions occurred.

Slight fever reactions occurred in 3 horses due to *Nuttallia equi.*

Experiment S. 4013 *of* 20.9.29. R. A. Alexander.

Object: To ascertain whether the virus will maintain its infectivity and propagate during passage through insusceptible animals, e.g. dogs.

Method: Inject dogs with fresh citrated blood from a reacting sheep. At 10-day intervals subinoculated into dogs for several generations and finally into susceptible sheep.

Result: After passage through 4 and 8 generations of dogs the virus was lost and produced no reaction in susceptible sheep. None of the dogs showed any febrile disturbance.

Artificial Methods of Transmitting Heartwater.

Experiment S. 240 *of* 10.12.28. G. de Kock.

Object: To test the efficacy of various modes of infection.

Method: Fresh citrated blood from a reacting sheep was used as indicated.

Result: 2 sheep—10 c.c. intrajugularly: Both reacted and both died.
2 ,, 10 c.c. subcutaneously: One sheep reacted and one sheep failed to react.
2 ,, 10 c.c. intraperitoneally: One sheep slight fever and one no reaction.
2 ,, 10 c.c. intratracheally: No reaction.
2 ,, 10 c.c. per os: No reaction.

All sheep which did not react proved susceptible on immunity test and all reactors or recovery proved immune.

Experiment S. 247 *of* 12.12.18. G. de Kock.

Object: To test the virulence of Heartwater blood in vivo before, during, and after a Heartwater reaction.

Method: Susceptible sheep injected with 10 c.c. virulent defibrinated blood intrajugularly as indicated.

Result: 2 sheep on 2nd day after injection of donor: Negative.
2 ,, at height of reaction: Both sheep reacted.
2 ,, after reaction (period not given): Negative.

Experiment S. 397 *of* 7.5.19. D. T. Mitchell.

Object: To retest different methods of artificial production of Heartwater.

Method: Subinoculations into susceptible sheep as indicated.

Result: 2 sheep—10 c.c. fresh citrated blood intraperitoneally: No reaction.
2 ,, 10 c.c. ,, ,, subcutaneously: No reaction.
2 ,, 10 c.c. ,, ,, intratracheally: No reaction.
1 ,, 10 c.c. ,, ,, intrajugularly: Reacted and died.

Experiment 457 *of* 7.6.19. D. T. Mitchell.

Object: To retest different methods of artificial production of Heartwater.

Method: As before.

Result: 1 sheep—10 c.c. fresh defibrinated blood intraperitoneally. No reaction.
1 ,, 10 c.c. ,, ,, intraperitoneally: Fairly severe reaction.
1 ,, 10 c.c. ,, ,, subcutaneously: Very mild reaction.
1 ,, 10 c.c. ,, ,, subcutaneously: Died on 10th day. No heartwater.

Experiment S. 2281 *of* 13.10.25. P. J. du Toit.

Object: To test the virulence of strain 860 by subcutaneous injection of 5, 10, 25, and 50 c.c. of virulent citrated blood from a reacting sheep.

Result: No reactions; and these sheep, when tested intrajugularly later, proved susceptible. Two controls injected intrajugularly reacted, not severely, and recovered.

*Experiment S.*2322 *of* 10.11.25. P. J. du Toit.

Object: To compare the reactions obtained by the intrajugular injection of various quantities of Heartwater virus.

Method: Susceptible sheep were injected with 0.1, 0.5, 1.0, 5.0 c.c. of fresh citrated blood. Three different strains of virus were used and with each amount 3 sheep were used.

Result: (*a*) Strain C. 860 gave reactions in 0.1, 0.5 and 5 c.c. amounts. No reaction with 1 c.c. One death occurred in 5 c.c. lot, making mortality 1 in 12.

(*b*) Snotsiekte strain gave reactions in all quantities, and death occurred after 0.5 c.c. (2 sheep) and 5.0 c.c. (1 sheep). Mortality 3 in 12.

(*c*) Vivax strain gave reactions with all quantities, and death occurred after 5 c.c., making the mortality 1 in 12.

Experiment S. 2411 *of* 11.2.26. P. J. du Toit.

Object: To note whether Heartwater can be transmitted by subcutaneous injection to calves. The animals injected intrajugularly will act as controls throughout.

Result:

Date.	Injected Into.	Injected From.	Method.	Result.
10/2/26	B. 1796	S. 14273	5 c.c. i.v.	Reacted to Heartwater and recovered.
10/2/26	B. 1229	S. 14273	5 c.c. subc.	No reaction.
10/2/26	B. 1198	S. 14273	5 c.c. subc.	No reaction.
27/2/26	S. 12205	B. 1796	5 c.c. i.v.	Reacted to Heartwater and died.
27/2/26	G. 14036	B. 1796	5 c.c. i.v.	Reacted to Heartwater and died.
29/3/26	S. 12204	B. 1796	5 c.c. i.v.	No reaction
29/3/26	S. 14320	B. 1796	5 c.c. i.v.	No reaction
27/2/26	B. 1229	B. 1796	5 c.c. i.v.	No reaction
20/3/26	S. 14349	B. 1229	5 c.c. i.v.	Heartwater reaction and recovered.
20/3/26	G. 14408	B. 1229	5 c.c. i.v.	Heartwater reaction and died.
13/3/26	B. 1198	S. 12205	5 c.c. i.v.	Heartwater reaction and died.
26/3/26	S. 11997	B. 1198	10 c.c. i.v.	Heartwater reaction and died.
26/3/26	S. 14341	B. 1198	10 c.c. i.v.	No reaction.
6/4/26	B. 1193	S. 14349	5 c.c. i.v.	Reacted to Heartwater and recovered.
6/4/26	B. 1194	S. 14349	5 c.c. i.v.	Reacted to Heartwater and recovered.
6/4/26	S. 12049	S. 14349	5 c.c. i.v.	Reacted to Heartwater and died.
6/4/26	S. 12207	S. 14349	5 c.c. i.v.	Reacted to Heartwater and died.
23/4/26	S. 8443	B. 1193	5 c.c. subc.	No reaction.
23/4/26	S. 14366	B. 1193	5 c.c. subc.	No reaction.
23/4/26	B. 1195	B. 1193	5 c.c. subc.	No reaction.
27/4/26	B. 1656	B. 1193	5 c.c. subc.	No reaction.
27/4/26	S. 14271	B. 1193	5 c.c. i.v.	Reacted to Heartwater and recovered.
23/4/26	S. 12007	B. 1194	5 c.c. subc.	Reacted to Heartwater and died.
23/4/26	G. 14593	B. 1194	5 c.c. subc.	No reaction.
23/4/26	B. 1196	B. 1194	5 c.c. subc.	No reaction.
27/4/26	B. 1223	B. 1194	5 c.c. i.v.	Reacted to Heartwater and died.
27/4/26	S. 14270	B. 1194	5 c.c. i.v.	Reacted to Heartwater and died.
11/5/26	S. 12068	S. 12007	5 c.c. i.v.	Reacted to Heartwater and died.
14/5/26	S. 12021	S. 12007	5 c.c. i.v.	Reacted to Heartwater and recovered.
24/5/26	S. 11425	S. 12007	5 c.c. subc.	No reaction.
28/5/26	S. 9208	S. 12068	5 c.c. i.v.	Reacted to Heartwater and died.
28/5/26	S. 8362	S. 12068	5 c.c. subc.	No reaction.
28/5/26	S. 8889	S. 12068	5 c.c. subc.	No reaction.

ABBREVIATIONS.

B. = Bovine.
G. = Goat.
S. = Sheep.
i.v. = Intravenously.
subc. = Subcutaneously.

Experiment S. 2428 *of* 20.2.26. P. J. du Toit.

Object: To note whether an intradermal injection of 0.5 c.c. blood will transmit Heartwater, the control to receive 5 c.c. intrajugularly.

Method: Intradermal injection made into the skin of the ear of a sheep.

Result: 0.5 c.c. blood intradermally: Negative.
5 c.c. blood intrajugularly: Reaction.

Experiment S. 4224 *of* 18.7.30. R. A. Alexander.

Object: To determine at what stage during the incubation period of the disease the blood becomes virulent.

Method: Two susceptible sheep were injected with Heartwater virus [intrajugularly (5 c.c.)]. Subinoculations were made from each of these two sheep into susceptible sheep daily until commencement of the febrile reaction in the donors.

Result: The first reactions obtained were those in sheep which received blood taken 24 hours prior to the commencement of the febrile reaction in the donors. All previous injections were negative and all subsequent injections positive. All animals that reacted recovered and proved immune subsequently and all non-reactors proved susceptible.

The Transmission by Ticks.

Experiment S. 1896 *of* 1.11.24. P. J. du Toit.

Object: To produce a case of Heartwater by means of ticks.

Method: Amblyomma hebraeum nymphae that had fed as larvae on a bovine during a Heartwater reaction were placed on scrotum of Sheep 8049 on 1.11.24.

Result: Febrile reaction commenced on 14.11.24 and remained high from 17-21.11.24, but returned to normal on 26.11.24 and the animal recovered.

Experiment S. 2036 *of* 9.3.25. P. J. du Toit.

Object: To produce cases of Heartwater by means of infected ticks and to infect a new lot of ticks.

Method: Usual. *Amblyomma hebraeum* nymphae used.

Result: The heifer on which the ticks were fed passed through a typical reaction after a period of incubation of 15 days and recovered. The diagnosis was confirmed by subinoculation into susceptible sheep.

Experiment 2459 *of* 23.3.26. P. J. du Toit.

Object: To ascertain whether *A. hebraeum* larvae that have been sucking blood from a calf during a reaction will transmit the disease when ground up and injected into sheep.

Method: A. hebraeum larvae when engorged on an animal suffering from Heartwater were ground up in saline and injected into two sheep, each of which received the equivalent of five ticks.

Result: No reaction was produced, and the sheep proved susceptible on subsequent immunity test.

Experiment 2494 *of* 10.11.26. P. J. du Toit.

Object: To transmit Heartwater by the intrajugular injection of crushed *Amblyomma hebraeum* nymphae infected as larvae.

Method: (*a*) 5 nymphae were ground up and injected into 2 sheep.
(*b*) 5 nymphae incubated for 24 hours and then ground up and injected.

Result: No reactions were produced, and the sheep were proved susceptible on immunity test.

Experiment S. 2883 *of* 31.12.26. P. J. du Toit.

Object: To transmit Heartwater by means of an emulsion of *A. hebraeum* nymphae and larvae which fed on Ox 1671 during a Heartwater reaction and were collected engorged on 15.12.26.

Method: The ticks were emulsified in two groups, one with and one without sand at intervals of 14 days and immediately injected into susceptible sheep intrajugularly, each sheep receiving the equivalent of 5 ticks.

Result:

14 days—
5 engorged larvae in 5 c.c. saline: No reaction.
5 ,, ,, 5 c.c. saline (with sand): No reaction.

28 days—
5 ,, ,, 5 c.c. saline: No reaction.
5 ,, ,, 5 c.c. saline (with sand): No reaction.

42 days—
5 nymphae in saline: No reaction.
5 ,, ,, (with sand): Slight thermal disturbance.[1]

56 days—
5 nymphae in saline: No reaction.
5 ,, ,, (with sand): No reaction.

70 days—
5 nymphae in saline: No reaction.
5 ,, ,, (with sand): No reaction.

84 days—
5 nymphae in saline: No reaction.
5 ,, ,, (with sand): Reaction.[2]

[1] Subinoculation in 2 susceptible sheep gave no reaction.
[2] Subinoculation in 2 sheep gave a good reaction which was carried out by further subinoculation.

Experiment S. 3338 *of* 14.12.27. P. J. du Toit.

Object: To ascertain whether males of *A. hebraeum* will transmit the disease.

Method: Males which had fed on a reacting bovine as nymphae were fed on goats.

Result: Goat 14593 infected on ears on 14.12.27. Heartwater reaction 26.12.27; died 31.12.27.

Experiment S. 3482 *of* 20.4.28. P. J. du Toit.

Object: To determine whether *A. marmoreum* is capable of transmitting the disease.

Method: Larvae fed on goats during the reaction and transmission to be attempted in the nymphal stage.

Result: Goats died before ticks were engorged. Hence valueless.

Experiment S. 3776 *of* 5.1.29. R. A. Alexander.

Object: To determine whether A. marmoreum is capable of transmitting the disease.

Method: The larvae hatched from eggs laid by an engorged female collected in Zululand. They were fed on Calf 2491 (infected with Heartwater) from 6.6.28-13.6.28, and after moulting on 10.9.28 were placed on 2 susceptible goats.

Result: No reaction and susceptibility of goats established on immunity test.

Experiment S. 3991 *of* 5.9.29. R. A. Alexander.

Object: To ascertain whether *A. nuttalli* is a transmitter of Heartwater.

Method: A. nuttalli batch 1092 were fed on Calf 3541 from 1.9.30 during the Heartwater reaction. These ticks, after moulting to nymphae, were fed on 3532 which did not react to Heartwater though fully susceptible. The nymphs on dropping were emulsified immediately and injected into two sheep, each sheep receiving the equivalent of two ticks.

Result: No reaction.

Exppriment 4177 *of* 16.5.30. R. A. Alexander.

Object: To study the transmission of Heartwater by ticks.

Method: *A. hebraeum* nymphae batch 1105 infected as larvae were fed on susceptible bovines.

Result: No reaction.

Ex. 2512, 2620, 2621, 2664, 2305. *Cf.* 13th and 14th Reports of D.V.S.

Retention of the Virus in the Tick.

Experiment 3320 *of* 1.12.27. P. J. du Toit.

Object: To ascertain (A) whether *A. hebraeum* infected as larvae and fed as nymphae on an insusceptible animal, e.g. the horse, will transmit the disease.

(B) Whether *A. hebraeum* nymphae infected as larvae will transmit Heartwater to a bovine.

(A) *Method:* *A. hebraeum* larvae batch 966 were infected by feeding as larvae on C. 1114 during a Heartwater reaction, were fed as nymphae on a horse and then, as adults, placed on Heifer 2039 on 1.12.27.

Result: C. 2039 commenced a slight reaction on 19.12.30, lasting till 25.12.27. On 24.12.27 subinoculations were made into 18311, 18313, both of which died of Heartwater.

(B) *Method:* *A. hebraeum* nymphae P. 928 infected as larvae were fed on 1803 on 1.12.27.

Result: C. 1803 showed a reaction from 17.12.27-25.12.27. On 28.12.27 subinoculations were made into S. 18330 and 18333, both of which reacted typically. C. 1803 and 2039 subsequently received 5 c.c. of blood from reacting goat 14456 on 7.1.27, and both failed to show any reaction.

Studies on Immunity.

Experiment S. 1888 *of* 11.11.24. P. J. du Toit.

Object: To determine whether animals immune against one strain of Heartwater can be reinfected with another strain.

Method: Sheep recovered from the Zontpansberg strain are infected with the Harding strain and vice versa.

Result:

Blood from.	Is injected into.	Which are immune against.	Result.
S. 8906 Zoutpansberg strain	6272	Harding strain.....	No reaction.
	8624	,, ...	,,
	9161	,, ...	,,
	7795	Zoutpansberg strain.....	,,
	9439	Control.....	Reaction.
S. 9348 Harding strain	5857	Zoutpansberg strain.....	Reaction.
	7710	,, ,, ...	No reaction.
	8864	,, ,, ...	,,
	8930	Harding strain.....	,,
	8735	Control.....	Reaction.

Experiment S. 2142 *of* 16.5.25. P. J. du Toit.

Object: To test the immunity of sheep and goats with a strain of virus different from the one the animals had been immunised with.

Method: 10 sheep immune to Z, C. 853, C. 850, Krugersdorp and Harding strains tested against 1024 strains.

Result: All the sheep were immune to 1024 strain, though a slight thermal disturbance was seen in a few instances.

Experiment S. 2143 *of* 16.5.25. P. J. du Toit.

Object: As Experiment S. 2142.

Method: 2 sheep that had recovered from reaction to Krugersdorp virus, 1 sheep to Z virus, 2 sheep to 860 virus, and 1 reactor to Harding virus were tested on their immunity to 860 virus. In addition were included 2 sheep which had not reacted to Zoutpansberg virus.

Result: The sheep that had not reacted previously possessed no immunity and passed through a severe reaction. All the other sheep failed to react except the one immune to the Harding strain, which passed through a mild reaction.

Experiment S. 2149 *of* 20.5.25. P. J. du Toit.

Object: As Experiment S. 2142.

Method: 4 sheep immune to Zoutpansberg strain, 2 sheep to Krugersdorp strain, and 2 sheep and 1 goat to 853 strain, were tested on their immunity to 860 strain.

Result: There were no reactions.

Experiment S. 2150 *of* 20.5.25. P. J. du Toit.

Object: As Experiment S. 2142.

Method: 2 sheep immune to 1024 strain, 1 sheep immune to 853 strain, and 1 sheep that had failed to react to Zoutpansberg strain were tested against 860 strain.

Result: The non-reactor to Zoutpansberg strain had a severe attack and died. The other sheep failed to react, but slight thermal disturbance was evident.

Experiment S. 3119 *of* 4.7.27. P. J. du Toit.

Object: To test the immunity of a number of sheep and goats which had recovered from infection with Snotsiekte strain (Experiment S. 2278).

Method: Inject the sheep intrajugularly with 5 c.c. of fresh citrated blood taken from a sheep reacting to the same strain.

Result:

Sheep.	In Experiment S. 2278.		Immunity Test.	
	Injected with.	Result.	Injected with.	Result.
10897...	5 c.c. blood from 16143	Negative	5 c.c. blood from 16188	Reaction.
15200...	,, ,,	,,	,, ,,	,,
15525...	,, ,,	Reaction	,, ,,	Negative.
15638...	,, ,,	,,	,, ,,	,,
15641...	,, ,,	Negative	,, ,,	Reaction.
16138...	,, ,,	,,	,, ,,	Reaction.
16200...	,, ,,	,,	,, ,,	Reaction. Died.
16203...	,, ,,	,,	,, ,,	Reaction.
16208...	,, ,,	,,	,, ,,	Reaction. Died.
16210...	,, ,,	,,	,, ,,	Reaction.
16212...	,, ,,	,,	,, ,,	Reaction. Died.
16213...	,, ,,	,,	,, ,,	Reaction.

Experiment S. 4020 *of* 30.9.29. R. A. Alexander.

Object: To determine—

(1) the duration of immunity in the recovered animal;
(2) whether the recovered animal remains a reservoir of the virus, and if so, for how long;
(3) whether reinfection of an immune animal causes its blood to become infective and, if so, for how long.

Method: (1) Subinoculation from reacting sheep at various times after the reaction which produced the immunity.

(2) Subinoculation into susceptible sheep after the first reaction.

(3) Subinoculation into susceptible sheep after attempted second reaction.

Result: When a sheep has reacted to a strain of Heartwater it will be immune to the same strain 62-156 days after infection though the immunity test may give a slight febrile reaction with no clinical symptoms. To a different or heterologous strain is not complete 143-156 days after infection. A marked febrile reaction may occur, without, however, any pathognomonic clinical symptoms of Heartwater, i.e. only symptoms of fever. Also 62-156 days after an infective dose sheep do not harbour the virus in 5 c.c. of blood.

Also, 31 days after an effective or ineffective second infection the animal does not harbour the virus in 5 c.c. of blood.

Experiment S. 4019 *of* 30.9.29. R. Alexander.

Object: To determine the duration and degree of immunity to homologous and heterologous strains of Heartwater virus.

Method: Infect animals with strains of virus by passage at the height of the reactions. Retail all recovered sheep and test their immunity against the homologous strain as well as against different strains.

Result: (1) There was no apparent dimiuntion in immunity 11 months after recovery as compared with animals recovered about 14 days.

(2) Of 34 sheep tested on immunity against the same strain, 2 died and 5 showed a severe reaction. Of 37 sheep tested on immunity against different strains, 5 died and 8 showed a severe reaction.

Experiment S. 4237 *of* 12.8.30. R. A. Alexander.

Cf. Longevity of the Virus.

Experiment S. 4140 *of* 11.3.30. R. A. Alexander.

Object: To ascertain whether sheep immunised against one strain of Heartwater are immune against all strains.

Method: Recovered sheep which had been used to keep the virus alive were tested for immunity against different strains of varying virulence.

Result: (1) A virulent strain produces no more immunity than a mild strain.

(2) After two positive reactions a third reinfection is not successful.

(3) After one reaction a second reaction frequently occurs on reinfection but is not fatal.

Duration of Immunity.

Experiment S. 447 *of* 30.6.19. D. T. Mitchell.

Object: To ascertain how long immunity lasts after recovery from an artificial attack of Heartwater.

Method: Recovered sheep injected intrajugularly with 10 c.c. of fresh citrated blood taken from an animal reacting to a different strain of virus.

Result: 1 sheep 14 weeks after recovery failed to react.
1 ,, 8 weeks after recovery showed a fairly severe reaction.

Active Immunisation.

Experiment S. 2353 *of* 7.12.25. P. J. du Toit.

Object: To immunise sheep against Heartwater.

Method: Sheep receive two successive injections of virus; the first subcutaneously and the second intrajugularly 14 days later. The controls are injected intrajugularly and they serve for the supply of the second virus on the 14th day.

Three different strains of virus and immunity is to be tested against the same virus; in each strain 10 sheep and 5 controls.

Result: Of the sheep first injected subcutaneously and 14 days later intrajugularly with the same strain of virus none died of Heartwater.

Strain 860—subcutaneously. 9 reacted + 1 negative. Controls: 4 reacted + 1 negative.

Snotsiekte—subcutaneously. 10 reacted. Controls: 1 died + 1 reacted + doubtful + 2 negative.

Vivax—subcutaneously. 6 reacted + 4 negative. Controls: 3 reacted + 2 doubtful.

Sheep which reacted to the first subcutaneous reaction were immune to the second intrajugular reaction; sheep which did not react were not immune, and appeared to have developed no partial immunity.

Experiment S. 2384 *of* 18.1.26. P. J. du Toit.

Object: To immunise sheep by means of virus injections.

Method: Sheep receive a first injection of 5 c.c. subcutaneously, followed 14 days later by a second injection of 5 c.c. intrajugularly. Strain 921 used.

Result: Thirty sheep received a first injection of 5 c.c. of blood from reacting Sheep 13879 subcutaneously, and 14 days later 5 c.c. intrajugularly of the same strain. One sheep, 11815, reacted to the subcutaneous injection recovered, and did not react to the intrajugular reaction. One sheep did not react to either the subcutaneous or intrajugular reaction. The remainder reacted only to the second injection, and of these 12 (60 per cent.) died. Of the 10 controls one was killed before the reaction started; of the remaining 9 one did not react and four of the 8 reactors died (50 per cent.).

All sheep which reacted and recovered were immune; non-reactors possessed no immunity.

Experiment S. 3790 *of* 15.1.29. R. A. Alexander.

Object: To attempt to immunise sheep by the subcutaneous injection of formalised material from spleen, liver, kidney, and brain.

Method: A sheep was killed just after the height of the febrile reaction. The spleen, liver, kidneys, and adrenals, and brain were removed with asceptic precautions, minced up, and isotonic saline added to make a 20 per cent. emulsion. This emulsion was shaken up well with beads. Formalin in a concentration of 0.1 per cent. commercial formalin was added, incubated for three days, and injections made in 5 c.c. amounts after seven days.

The experiment was duplicated, using the supernatant fluid injected intrajugularly.

Result: There was no reaction to the injection of formalised material in 10 sheep, and on immunity test all sheep reacted normally.

Experiment S. 3991 *of* 5.9.29. R. A. Alexander.

Object: To immunise bovines against Heartwater by the injection of a formalised emulsion of infective ticks.

Method: A. hebraeum larvae batch 1109 were infected by feeding on 3515, 3528, and 3529, which were reacting to Heartwater as a result of intravenous injection of blood from reacting Sheep 23042. After moulting, some of these ticks were fed on Bovine 2998 for 48 hours (1 tick for 60 hours). Heartwater was produced, the disease being definitely diagnosed by subinoculation into 2 sheep, 24702 and 24703, both of which died of Heartwater confirmed by demonstration of *Rickettsia* in the tissues. The ticks were removed after feeding for the time stated, and on emulsion made by grinding with sand and isotonic saline. Sheep were injected with this emulsion, each of 2 sheep receiving the equivalent of 1 and 2.5 ticks.

Result: Heartwater was not produced, though the sheep were susceptible as shown by severe reaction on immunity test.

Experiment S. 4300 *of* 11.11.30. R. A. Alexander.

Object: To attempt to immunise sheep by the injection of formalised emulsion of infective ticks (*A. hebraeum*).

Method: Batch 1105 adults infected by feeding on Sheep 25124 (Experiment 4192) were activated by feeding on Bovine C. 3501. This animal subsequently developed Heartwater but no engorged adult ticks were collected for various reasons.

Batch 1154 were fed on a reacting bovine as nymphs. As adults they were fed on C. 3522, which did not develop Heartwater. The ticks on engorgement were emulsified portion formalised in a dilution of 1 part of formalin to 1,000 parts of a 20 per cent. tick emulsion.

Result: Two sheep died of glottis oedema as a result of intravenous injection of unformalised emulsion which contained the equivalent of 0.5 and 0.25 tick. None of the remaining sheep reacted, and all proved fully susceptible on immunity test. Owing to the above result the formalised emulsion was not injected.

Experiment S. 4141 *of* 17.3.30. R. A. Alexander.

Object: To immunise cattle and sheep against Heartwater by the injection of formalised tick virus.

Method: A. hebraeum nymphae batch 1134 Aa infected as nymphae by feeding on a bovine during a Heartwater reaction, did not produce the disease when fed on C. 3511, which animal later did not react to 10 c.c. of virulent blood intrajugularly. The engorged nymphae were immediately emulsified with sand and saline to form a 20 per cent. emulsion. Portion of this was injected into 2 sheep intrajugularly, the dose being equivalent to 5 ticks; 1 died almost immediately of protein shock and glottis oedema, 1 developed Heartwater and died. The formalised material (1 part of formalin to 1,000 parts emulsion) was injected into sheep.

Result:

Material.	Sheep.	Result.	Immunity Test.	
			Interval.	Result.
1 c.c. formalised virus = 1 tick.	25508	No reaction	23 days	Severe reaction. Recovered.
	25657	,,	23 ,,	Died Heartwater.
2·5 c.c. formalised virus = 2·5 ticks.	25687	,,	23 ,,	,, ,,
	25708	,,	23 ,,	,, ,,
5·0 c.c. formalised virus = 5 ticks.	25685	,,	23 ,,	,, ,,
	25795	,,	23 ,,	,, ,,
10·0 c.c. formalised virus = 10 ticks	25518	,,	23 ,,	,, ,,
	25814	Died shock	23 ,,	,, ,,
	63543	No reaction	23 ,,	No reaction.

Passive Immunisation.

Experiment S. 1394 *of* 5.4.23. P. J. du Toit.

Object: To produce Heartwater serum by the hyperimmunisation of immune cattle with large quantities of Heartwater virus.

Method: Immune cattle received up to 3 litres of virulent defibrinated blood subcutaneously and intrajugularly repeated on three occasions at 5 to 7 day intervals. The blood was taken when required and serum syphoned off after coagulation.

Experiment S. 1563 *of* 9.4.24. P. J. du Toit.

Object: To determine the interval after the injection of virus when Heartwater serum will stop the reaction.

Method: Goats were used in the experiment and injections were made intrajugularly.

Result:

Day 0.	Day 1.	Day 2.	Day 3.	Day 5.	Result.
10 c.c. virus	20 c.c. serum	—	—	—	Reaction. Recovered.
10 ,,	—	20 c.c. serum	—	—	,, Died. ,, ,,
10 ,,	—	—	20 c.c. serum	—	,, Recovered. ,, Died. ,, ,,
10 ,,	—	—	—	20 c.c. serum	,, Recovered. ,, ,,
10 ,,	Controls.....		.....	.....	Reaction. Recovered. ,, ,,

Experiment S. 1653 *of* 13.5.24. P. J. du Toit.

Object: To immunise goats against Heartwater.

Method: Sheep and cattle were hyperimmunised by the repeated injection of large quantities of virulent blood. Goats received 50 c.c. of hyperimmune serum and different individuals received virus simultaneously or on successive days up to 10 days. Serum injected subcutaneously and virus intrajugularly.

Result: Six out of ten goats died of Heartwater and the remaining four recovered after a very severe reaction.

Experiment S. 2192 *of* 6.7.25. P. J. du Toit.

Object: To study the effect of hyperimmune serum on Heartwater virus injected—

(*a*) 24 hours before virus in 25 and 50 c.c. amounts intrajugularly and subcutaneously;
(*b*) simultaneously in same amounts as (*a*);
(*c*) 24 hours after virus.

Virus strain 853 used on sheep.

Result: The serum had no effect upon the course of the disease whether injected 24 hours before, 24 hours after, or simultaneously with the virus subcutaneously or intravenously. When the virus was given subcutaneously no reaction followed. Recovered animals were immune. Non-reactors were not immune.

Experiment S. 2196 *of* 7.7.25. P. J. du Toit.

Object: To study the effect of hyperimmune serum on Heartwater virus (strain 860) injected—

(*a*) 24 hours before virus in 25 and 50 c.c. amounts intrajugularly and subcutaneously;
(*b*) simultaneously in like quantities;
(*c*) 24 hours after virus in like quantities.

Method and Result: Identical with S. 2192 above.

Experiment S. 2211 *of* 30.7.25. P. J. du Toit.

Object, Method, and Results: Identical with S. 2192 above.

Experiment S. 2271 *of* 28.9.25. P. J. du Toit.

Object: To test the virulence of Heartwater virus strain 860 by subcutaneous injection of small and large quantities (5 and 20 c.c.) and by simultaneous intrajugular and subcutaneous injections of hyperimmune serum, and subcutaneous and intrajugular injection of virus.

Method: As outlined under " object " control sheep 3 received 5 c.c. intrajugularly.

Result: All 3 controls reacted; 2 recovered and 1 died. Three sheep received 10 c.c. serum and 5 c.c. virus simultaneously intrajugularly; all 3 reacted and recovered. Ten sheep received 10 c.c. serum and 5 c.c. virus simultaneously subcutaneous; no reactions, and on immunity test by intrajugular injection 8 reacted and recovered, while 2 did not react.

Five sheep received 5 c.c. virus subcutaneously; none reacted; on immunity test 4 reacted and recovered and one failed to react. Five sheep received 10 c.c. serum intrajugularly and 5 c.c. virus subcutaneously simultaneously; 1 reacted and 4 failed to react. On immunity test all but the reactor gave a definite reaction but recovered.

Five sheep received 20 c.c. serum intrajugularly and 5 c.c. virus subcutaneously simultaneously. None reacted. On subsequent immunity test 3 reacted and 2 did not react.

Experiment S. 2339 *of* 23.11.25. P. J. du Toit.

Object: To test the effect of 20 c.c. of hyperimmune serum injected subcutaneously on intrajugularly and simultaneously virus (3 strains).

Result: All 15 sheep injected with strain 860 reacted. Two of those which received serum intrajugularly died, and also two of those which received serum subcutaneously. No controls died. In the " Snotsiekte " strain group the result was similar. One sheep died in each of the serum groups, none amongst the controls. In the " vivax " strain group one sheep which received serum intrajugularly died, and 2 controls.

Summary: Two controls out of 15 died, and 7 out of 30 which received serum died. The serum, therefore, appears to have no protective influence.

Drug Treatment of Heartwater.

Experiment S. 1563 *of* 10.10.1923. P. J. du Toit.

To attempt to cure Heartwater in goats and cattle by means of various treatment.

Tryparsamide cattle: 8 c.c. of a 25 per cent. solution (.025 per kilo); 3 days later, .16 c.c. of 25 per cent. solution; 3rd and 4th day later, 50 c.c. of Heartwater serum. Result: Died of Heartwater.

Tryparsamide goat, intrajugularly: 6 c.c. of 25 per cent. solution=0.05 gm. per kilogram. Result: Died of Heartwater.

Tryparsamide goat, intrajugularly: 6 c.c. of 25 per cent. solution=.0375 gm. per kilogram and 2 days later 12 c.c.=.075 gm. per kilogram. Result: Died of Heartwater.

Tryparsamide goat, intrajugularly: 5 c.c. of 25 per cent. solution=.05 gm. per kilogram and 2 days later 9 c.c.=.075 gm. per kilogram. Result: Died of Heartwater.

Tryparsamide goat, intrajugularly: 6 c.c. of 25 per cent. solution=.04 gm. per kilogram. Result: Died of Heartwater.

T. 10.	Goat intraj.	1·8	gm. in	20	c.c. water	=0·05 gm. per kilo.	Result: Died of Heartwater.	
	,, ,,	1·0	,,	10	,,	=0·03 gm. per kilo.	Result: Died of Heartwater.	
	,, ,,	1·0	,,	10	,,	=0·03 gm. per kilo. repeated on second day.	Result: Died of Heartwater. All controls.	
M. 201.	,, ,,	2	,,	10	,,	=0·04 gm. per kilo.	Result: Died of Heartwater.	
M. 203.	,, ,,	1	,,	10	,,	=0·025 gm. per kilo.	Result: Died of Heartwater.	
M. 204.	,, ,,	0·5	,,	10	,,	=0·03 g.m per Kilo. preceded the day before by 10 c.c. serum.	Result: Died few minutes after injection.	
M 205.	,, ,,	0·8	,,	20	,,	=0·025 gm. per kilo.	Result: Died of Heartwater.	
M. 206.	,, ,,	1	,,	20	,,	=0·025 gm. per kilo.	Result: Died within few minutes.	
T. 13.	,, ,,	0·75	,,	10	,,	=0·03 gm. per kilo.	Result: Died	
T. 11.	,, ,,	1	,,	10	,,	=0·03 gm per kilo. repeated on second day.	Result: Died of Heartwater.	
T. 12.	,, ,,	1	,,	10	,,	=0·03 gm. per kilo. repeated on second day.	Result: Died of Heartwater.	Two controls.
T. 13.	,, ,,	1	,,	10	,,	=0·03 gm. per kilo. repeated on second day.	Result: Died of Heartwater.	

Bayer 205.	Goat	1	gm. in	10	c.c. water	Result: Recovered. Weight: 72 lb. =0·03 gm. per kilo.	
	,,	1	,,	10	,,	Result: Recovered. Weight: 80 lb.	
	,,	0·75	,,	10	,,	Result: Died. Weight: 99 lb.	
	,,	0·5	,,	10	,,	Result: Died. Weight: 96 lb.	
	,,	1	,,	10	,,	Result: Recovered. Weight 112 lb.	
	,,	2	,,	10	,,	Result: Recovered. Weight: 79 lb.	Of
	,,	1	,,	10	,,	Result: Recovered. Weight: 65 lb.	controls.
	,,	1	,,	10	,,	Result: Died. Weight: 73 lb.	
	,,	1	,,	10	,,	Result: Died. Weight not given.	4 recovered.
	,,	0·75	,,	10	,,	Result: Died. Weight not given.	
	,,	0·75	,,	10	,,	Result: Died. Weight: 60 lb.	
	,,	0·50	,,	10	,,	Result: Recovered. Weight: 108 lb.	1
	,,	0·50	,,	10	,,	Result: Recovered. Weight 70 lb.	died.
	,,	1·0	,,	10	,,	Result: Recovered. Weight not given	
	,,	1·0	,,	10	,,	Result: Recovered. Weight not given.	
	,,	0·75	,,	10	,,	Result: Died. Weight not given.	
	,,	0·75	,,	10	,,	Result: Died. Weight 104 lb.	

Goat	10	gm. subcut.	in	60	c.c. water.	Result: Died same day.	
,,	10	,,	,,	20	c.c. water.	Result: Died later.	
,,	10	,,	,,	50	,, ,,	,, ,,	Of controls 4 died 1 recovered.
,,	10	,,	,,	30	,, ,,	,, ,,	
,,	10	,,	,,	60	,, ,,	,, ,,	
,,	10	,,	,,	10	,, ,,	,, ,,	
,,	10	,,	,,	20	,, ,,	,, ,,	

Experiment S. 4228 *of* 24.6.30. R. A. Alexander and A. Malan.

Object: To ascertain the changes in amount of inorganic calcium in the blood of sheep during a Heartwater reaction.

Method: Infect 3 sheep with Heartwater by intrajugular injection of 5 c.c. of fresh citrated blood from a reacting sheep and determine the amount of calcium present every alternate day from the time of injection.

Results:

	Sheep 28223.	Sheep 28209.	Sheep 28408.
26/6/30	9·1 mgm. %	9·3 mgm. %	8·4 mgm. %
28/6/30	8·6 ,,	8·8 ,,	7·9 ,,
30/6/30	8·4 ,,	8·3 ,,	7·5 ,,
2/7/30	10·0 ,,	9·7 ,,	10·2 ,,
4/7/30	9·3 ,,	8·9 ,,	9·5 ,,
7/7/30	9·0 ,,	8·8 ,,	9·6 ,,
9/7/30	8·1 ,,	8·0 ,,	9·4 ,,
11/7/30	Dead.	Dead.	9·5 ,,

Experiment S. 4212 *of* 4.7.30. R. A. Alexander and G. Pfaff.

Object: To ascertain the effect of formalin as a curative for Heartwater.

Method and Results:

D.O.B. Number.	Infected from 4/7/30.	Reaction.	Treatment.	Remarks.
Non reactor 3522	S. 28199, 28355, 28422	No reaction.....	—	—
Control 3539	,, ,,	Slight reaction from 16th to 23/7/30. No clinical symptoms and maximum temperature 103·6°	—	Recovered without any treatment.
,, 3543	,, ,,	Slight reaction from 12 to 26/7/30. No clinical symptoms, maximum temperature 103·8.°	—	Recovered without treatment.
,, 3637	,, ,,	Severe reaction from 16–24/7/30. Severe clinical symptoms	Treated on 22/7/30 on 6th day of reaction with 2 c.c. formalin in 10 c.c. saline and showed immediate improvement and recovery	The formalin appeared to bring down temperature at once.
,, 3638	,, ,,	Severe reaction from 16–24/7/30. Severe clinical symptoms	Treated on 24/7/30 on 8th day of reaction with 3 c.c. formalin in 15 c.c. saline and showed immediate improvement and recovery	Recovery under treatment.
,, 3668	,, ,,	Reaction commenced on 17/7/30. Very severe with marked clinical symptoms	Treated on 24/7/30 on 7th day of reaction with 3 c.c. formalin in 15 c.c. saline. Immediate improvement	Although the temperature dropped within 48 hours of treatment for four weeks there were temperature remissions but no clinical Symptoms.
,, 3669	,, ,,	Reaction commenced 29/7/30 very severe and animal died 9/8/30	—	Control.
,, 3682	2692 on 15/7/30.....	Reaction as 3669. Severe with marked clinical symptoms	8th day of reaction 3 c.c. formalin in 15 c.c. saline. 9th day reaction 3 c.c. formalin in 15 c.c. saline.	Marked improvement within 24 hours of second injection resulting in complete recovery.
,, 3684	2696 on 15/7/30.....	Reaction as 3669 severe with clinical symptoms not well marked	No treatment.....	Animal gradually recovered with slight irregularities in temperature for nearly three weeks.
,, 3686	28199, 28355, 28422..	Slight reaction with no marked clinical symptoms lasting for over a month	No treatment.....	Spontaneous recovery but reaction prolonged.
,, 3694	2696 on 15/7/30.....	Slight reaction from 29/7/30, no marked symptoms, reaction prolonged for over a month	No treatment.....	Spontaneous recovery but reaction prolonged.
,, 3695	Sheep infection.....	Marked reaction commenced 18/7/30 with marked clinical symptoms	On 7th day of reaction treated with 3 c.c. formalin in 15 c.c. saline	Marked improvement in 48 hours and recovery.

Experiment S. 4212—Continued.

D.O.B. Number.	Infected from 4/7/30.	Reaction.	Treatment.	Remarks.
Control 3685	S. 28199, 28355. 28422	A fairly severe reaction commenced on 12/7/30. This decreased in severity on 18 and 19/7/30 when there was an acute remission with marked clinical symptoms	3 c.c. formalin in 15 c.c. saline on 12th day of reaction	Died. Treatment was undertaken when animal was practically *in extremis*.
,, 3696	2696 on 15/7/30.....	Reaction commenced on 29th with no definite clinical symptoms. Severe remission in 4/8/30 and treatment commenced when animal was down on 5/4/30	8th day of reaction 3 c.c. formalin in 15 c.c. saline intraj. 9th day of reaction 100 c.c. 5 per cent. Ca. lactate+3 c.c. formalin in 15 c.c. saline intraj. 10th day of reaction 100 c.c. 5 % Mag. citrate +3. c.c. formalin in 15 c.c. saline intraj.	Treatment had no appreciable influence. Animal died.
,, 3699	S. 28199, 28355, 28422	Reaction commenced on 16–26th with well marked clinical symptoms, followed by several slight remissions and irregular temperature for about one month	—	Animal recovered without treatment though the course of the disease was very prolonged.
,, 3701	2696 on 15/7/30.....	Reaction commenced on 28/7/30. Temperature never above 105 and clinical symptoms slight. Animal collapsed on 5/8/30	3 c.c. formalin in 15 c.c. saline when animal was *in extremis*. Died.	Treatment was obviously hopeless when undertaken.
,, 3701	2696 on 15/7/30.....	Reaction commenced on 29/7/30. Clinical sypmtoms which were severe only developed later on 4/8/30	6th day of reaction 3 c.c. formalin in 15 c.c. saline. 7th day of reaction 100 c.c. 5 % Ca lactate +3 c.c. formalin in 15 c.c. saline. 8th day of reaction 100 c.c. 5 % Mag. citrate + 30 c.c. formalin in 15 c.c. saline	Treatment had no apparent effect on the course of the disease.
,, 3713	2696 on 15/7/30.....	Reaction commenced on 29th, progressed rapidly and with advent of severe clinical symptoms. Died.	—	Died.

Of 8 animals injected with C. 2696 strain all 8 showed a severe reaction. Of these four were retained as controls. Of the controls two died and 2 recovered, though recovery was associated with a prolonged reaction which lasted several weeks.

Of the 4 treated animals 3 died, of which 1 was a hopeless case when treatment was commenced, and the fourth animal made a rapid recovery.

Of 10 animals injected with 10 c.c. intraj. from 3 reacting sheep (blood pooled), 1 did not react, 2 showed only a febrile reaction and the remaining 7 showed severe clinical symptoms in addition. Four animals were kept as controls, the two with no clinical symptoms and two with well marked clinical symptoms. All recovered, though the course of the disease was much prolonged. Of the five treated animals one died (treatment commenced *in extremis*), 3 showed marked response to treatment, and 1 could not be differentiated from the recovered control as regards prolonged reaction.

In spite of the apparent poor results it is concluded that, formalin is a valuable therapeutic agent when treatment is commenced early as shown by the prompt recovery with disappearance of very severe clinical symptoms in 3 animals.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

An Attempt to Attenuate the "Virus" of Heartwater by Passage.

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Theiler (1908) and, more recently, Du Toit (1929) have shown that the virus of "bluetongue" (catarrhal fever) of sheep can be attenuated by the simple process of subinoculating the blood of infected sheep. After the tenth or eleventh passage the virus which, at first, may have produced fatal results, becomes so attenuated that it can, with safety, be used as a vaccine.

The hope was entertained that a similar procedure might lead to an attenuation of the "virus" of heartwater and that this might provide a method of preventive inoculation. There appeared to be some justification for such a hope since a very low mortality had often been observed in animals inoculated with heartwater blood.

Experiments were accordingly undertaken with the object of testing the influence of repeated subinoculations on the virulence of heartwater virus.

The first experiment was started in April, 1925, and continued till January, 1926, during which time the strain was carried through twenty generations.

Meanwhile a second experiment had been started in August, 1925. In this case the process of subinoculation was continued for over five years through more than one hundred generations.

It may be stated at once that both experiments yielded practically negative results in so far as the production of a suitable vaccine was concerned. Nevertheless it is considered advisable to record briefly a few details of these experiments, since important information was obtained regarding the variability and general behaviour of the heartwater virus.

OBJECT OF THE EXPERIMENTS.

To determine whether it was possible to attenuate the " virus " of heartwater by repeated subinoculations through a large number of generations of susceptible animals, and whether such an attenuated strain of virus could be used to immunize animals against heartwater.

METHOD.

Without exception *blood* was used for the transmission of the disease from one generation to the next. The blood was taken from an animal during the reaction, usually at the height of the reaction, but sometimes at the beginning, and at other times at the end of the reaction. The blood was mixed with 10 per cent. citrate solution to prevent coagulation and was injected practically immediately into the animals to be infected. The *intrajugular* mode of injection was invariably chosen, since the subcutaneous injection was known, from extensive experience, to give unreliable results.

The large majority of animals used in these experiments were *sheep,* although a few goats were sometimes included and occasionally also a few cattle. Since the difference in susceptibility to heartwater between these classes of animals is not very great, it is considered unnecessary in the following pages to give details in regard to the class of animal used.

The animals used in the experiments were stabled. For this reason it may be assumed that the mortality throughout the experiments was somewhat lower than it would have been, had the animals been allowed to run in the open.

EXPERIMENT 1 (S. 2110).

The strain used in this experiment was obtained from a heifer (No. 860) which had contracted a natural infection of heartwater. Blood of this heifer was injected into a sheep and a goat to confirm the diagnosis. Both animals recovered after a comparatively mild reaction.

The idea then occurred that this strain of virus might prove to be naturally attenuated and consequently it was decided to continue the subinoculations through a series of generations.

The *results* are briefly indicated below:—

Table 1.

1st	generation,	2	animals injected,	0	deaths.
2nd	,,	4	,, ,,	4	,,
3rd	,,	4	,, ,,	0	,,
4th	,,	2	,, ,,	0	,,
5th	,,	2	,, ,,	1	death.
6th	,,	2	,, ,,	1	,,
7th	,,	14	,, ,,	2	deaths.
8th	,,	2	,, ,,	0	,,
9th	,,	2	,, ,,	1	death.
10th	,,	11	,, ,,	1	,,
11th	,,	11	,, ,,	0	deaths.
12th	,,	2	,, ,,	0	,,
13th	,,	3	,, ,,	1	death.
14th	,,	3	,, ,,	0	deaths.
15th	,,	31	,, ,,	1	death.
16th	,,	22	,, ,,	0	deaths.
17th	,,	19	,, ,,	1	death.
18th	,,	10	,, ,,	4	deaths.
19th	,,	4	,, ,,	0	,,
20th	,,	2	,, ,,	2	,,

Discussion of Results.

It will be noticed that after the initial favourable start (second generation, no death), the strain proved to be rather virulent (third generation, all animals died). Nevertheless the subinoculations were continued, and after a time it actually seemed as if an attenuation had taken place. To indicate this it is instructive to group the results as follows:—

1st to 10th generations, 45 animals, 10 deaths (22.2 per cent.)

11th to 17th generations, 91 animals, 3 deaths (3.3 per cent.)

Such a conclusion would, however, have been premature, as was proved by the very next generation, when 4 out of 10 sheep died, and, perhaps, more convincingly still, in the twentieth generation when both the injected sheep succumbed to the disease.

At this stage the strain seemed to be at least as virulent as it was at the beginning, and the experiment was abandoned.

Conclusion.

In the course of twenty subinoculations a strain of heartwater virus did not seem to have become attenuated in any way.

EXPERIMENT 2 (S. 2278).

In this case the strain originated from a calf (No. 1145) which had been injected with blood obtained from an ox suffering from "snotsiekte." The calf eventually died of the disease, but, before death, blood was taken and injected into two sheep to determine whether heartwater was present as a complicating factor. The reactions in these two sheep were somewhat inconclusive and further subinoculations were carried out to confirm the diagnosis. Finally the subinoculations were continued in the hope of obtaining an attenuated ("vaccine") strain of heartwater virus.

Many interesting data were collected in the course of this experiment and will be utilized elsewhere. For our present purpose a very brief statement of the *results* will suffice:—

TABLE 2.

No. of Generations.	Date of Injection.	No. of Animals Injected.	No. of Reactions.	No. of Deaths.	Remarks.
1	13. 8.25	2	1	1	One doubtful reaction.
2	20. 8.25	2	2	1	
3	2. 9.25	3	3	0	
4	16. 9.25	7	4	2	In this and other cases all the injections were *not* carried out on the same day; the point, however, is unimportant for the purposes of this table.
5	28. 9.25	14	11	2	
6	9.10.25	5	5	1	
7	26.10.25	2	2	0	
8	9.11.25	3	3	3	
9	21.11.25	5	3	0	
10	7.12.25	5	2	1	In some of the injected animals the reactions were doubtful.
11	25.12.25	2	2	1	
12	4. 1.26	2	2	1	
13	18. 1.26	2	2	0	
14	4. 2.26	4	4	2	
15	20. 2.26	3	3	1	
16	6. 3.26	2	2	2	
17	20. 3.26	2	1	1	
18	30. 3.26	2	2	0	
19	9. 4.26	2	2	0	
20	22. 4.26	2	1	0	
21	5. 5.26	2	2	1	
22	18. 5.26	2	2	2	
23	29. 5.26	2	2	0	
24	8. 6.26	2	2	1	
25	21. 6.26	2	2	0	
26	6. 7.26	2	2	0	
27	21. 7.26	2	2	0	
28	3. 8.26	2	1	1	
29	16. 8.26	2	2	1	
30	28. 8.26	2	2	0	
31	13. 9.26	2	2	1	
32	27. 9.26	2	2	1	
33	9.10.26	2	2	0	
34	23.10.26	2	2	0	
35	8.11.26	2	2	0	
36	26.11.26	3	3	0	
37	10.12.26	2	2	1	
38	21.12.26	2	2	0	
39	3. 1.27	2	2	0	
40	14. 1.27	3	2	0	
41	27. 1.27	3	3	0	
42	7. 2.27	4	4	0	
43	25. 2.27	2	2	0	No deaths for 6 generations.
44	11. 3.27	2	2	1	
45	23. 3.27	4	4	1	
46	10. 4.27	7	6	1	

TABLE 2—*Continued.*

No. of Generations.	Date of Injection.	No. of Animals Injected.	No. of Reactions.	No. of Deaths.	Remarks.
47a	5. 5.27	6	6	3	From the 47th to 67th generations the sub-inoculations were carried out in two parallel series, i.e. two sub-strains were maintained separately. They will be marked a and b.
b	25. 5.27	12	4	0	
48a	26. 5.27	2	2	1	
b	16. 6.27	2	2	0	
49a	15. 6.27	2	2	1	
b	6. 7.27	2	2	1	
50a	6. 7.27	2	2	0	
b	26. 7.27	2	2	1	
51a	30. 7.27	7	5	1	
b	10. 8.27	4	4	1	
52a	19. 8.27	2	2	2	
b	29. 8.27	4	3	2	
53a	7. 9.27	2	2	0	
b	17. 9.27	3	2	0	
54a	1.10.27	5	5	0	
b	2.10.27	2	2	2	
55a	19.10.27	4	4	3	
b	25.10.27	3	2	1	
56a	4.11.27	2	2	2	
b	12.11.27	2	2	0	
57a	21.11.27	2	2	1	
b	30.11.27	2	2	0	
58a	5.12.27	2	2	1	
b	9.12.27	2	2	1	
59a	17.12.27	2	2	1	
b	26.12.27	2	2	2	
60a	5. 1.28	2	2	2	
b	10. 1.28	2	2	1	
61a	19. 1.28	2	2	0	
b	23. 1.28	2	2	0	
62a	1. 2.28	2	2	0	
b	8. 2.28	2	2	0	
63a	18. 2.28	2	2	1	
b	23. 2.28	2	2	1	
64a	3. 3.28	2	2	1	
b	7. 3.28	2	2	1	
65a	16. 3.28	2	2	2	
b	25. 3.28	2	2	0	
66a	31. 3.28	2	2	1	
b	10. 4.28	2	2	0	
67a	16. 4.28	2	2	1	This sub-strain appeared to be so virulent that further sub-inoculations were discontinued.
b	25. 4.28	2	2	0	
$68b_1$	12. 5.28	2	2	0	Two sub-strains were again maintained for some time.
b_2	18. 5.28	2	2	0	
$69b_1$	28. 5.28	2	1	0	
b_2	1. 6.28	2	2	0	
$70b_1$	18. 6.28	2	2	0	
b_2	18. 6.28	21	17	4	19 of these animals were cattle.
$71b_1$	5. 7.28	4	2	0	
b_2	13. 7.28	4	2	0	
$72b_1$	25. 7.28	4	3	0	
b_2	2. 8.28	3	0	0	This sub-strain seemed to have died out and was discontinued.
$73b_1$	7. 8.28	6	4	1	

TABLE 2—*Continued.*

No. of Generations.	Date of Injection.	No. of Animals Injected.	No. of Reactions.	No. of Deaths.	Remarks.
74	27. 8.28	4	4	2	
75	12. 9.28	4	4	2	
76	27. 9.28	2	2	0	
77a	16.10.28	4	4	1	Again two sub-strains were maintained.
b	4.10.28	2	2	0	
78a	5.11.28	4	2	0	
b	24.10.28	2	2	0	
79a	12.12.28	6	3	0	
b	9.11.28	4	2	0	
80a	29.12.28	4	2	1	
b	24.11.28	4	0	0	
81a	14. 1.29	4	3	2	
b	27.11.28	2	1	0	
82a	29. 1.29	2	2	0	
b	3.12.28	4	0	0	
83a	14. 2.29	2	1	0	
b	7.12.29	2	0	0	Sub-strain b had evidently died out (see page 159) and was discontinued.
84a	28. 2.29	2	2	1	
85	14. 3.29	2	2	0	
86	27. 3.29	2	2	2	These were the last deaths in this series of sub-inoculations.
87	10. 4.29	2	2	0	
88	19. 4.29	4	4	0	
89	6. 5.29	2	2	0	
90	21. 5.29	2	2	0	
91	3. 6.29	2	2	0	
92	19. 6.29	4	2	0	
93	10. 7.29	4	3	0	
94	25. 7.29	4	3	0	
95	17. 8.29	4	1	0	
96	31. 8.29	7	2	0	At this stage and subsequently some of the reactions were so mild that it is difficult to give definite numbers in this table.
97	23. 9.29	6	3	0	
98	14.10.29	6	3	0	
99	25.10.29	22	12	0	
100	12.11.29	6	3	0	
101	29.11.29	8	5	0	
102	18.12.29	4	1	0	
103	7. 1.30	10	4	0	

It should be added immediately that the subinoculations were continued for another 10 months through a further 21 generations. However, it was found afterwards that the animals used in these later inoculations had become infected with *Trypanosoma congolense*. This infection was probably introduced through an ox used in the 103rd generation and it was considered therefore that all subsequent inoculations should be discarded for the purposes of our present experiment.

Although this accidental contamination of the strain was to be regretted, we considered ourselves fortunate that it occurred at a stage when the experiment was practically completed. As a matter of fact for a considerable time it was our intention merely to continue until the 100th generation had been reached.

Discussion of Results.

From the above table it will be evident at once that the rate of mortality amongst the inoculated animals was by no means constant. It will be noticed that periods of fairly severe mortality alternated with periods when, for several generations, there was no mortality at all. Thus, for six generations, from the 38th to the 43rd, there were no deaths, but this was followed by a period of fairly heavy mortality.

In the above table 460 animals are enumerated and of these 85 died of heartwater (18.5 per cent.). If we divide the experiment into four periods, the mortality rate works out as follows: —

Table 3.

Generations.	No. of Animals.	No. of Deaths.	Percentage.
1— 25	81	23	28·4
26— 50	84	15	17·9
51— 75	144	40	27·8
76—103	151	7	4·6

It would thus seem that there was no attenuation up to the 75th generation, and that a marked attenuation was brought about from this point onward.

But even this conclusion is open to criticism. Looking at the table the impression is gained that, by the time the 90th generation was reached, the strain had lost its virulence completely. That this was not the case was borne out by the following observation: In a separate experiment (S. 3870) a number of sheep (44) had been injected with varying quantities of different tissues of infected animals to determine the concentration of heartwater virus in these organs. Some of the sheep reacted and others did not. All the sheep were thereupon injected intrajugularly with 5 c.c. blood of sheep No. 16141 of the 90th generation of our experiment. The majority of the sheep reacted and 11 out of the 44 died, thus showing that the blood in the 90th generation was by no means avirulent.

In spite of this somewhat surprising experience there can be no doubt that the strain had become attenuated towards the end of the experiment. As a matter of fact, during the last 10 generations or so the majority of the animals injected did not show a definite reaction at all. At that stage the danger of losing the strain altogether became very real.

At first sight it would appear, therefore, as if the object of the experiment had been attained, namely the production of an attenuated

strain of heartwater virus. But it should be remembered that the ultimate object of the experiment was to find a strain which could be used as a vaccine for the immunization against heartwater. That this latter object had not been achieved by any means, may be deducted from the following facts:—

In the 98th, and again in the 102nd generation, an attempt was made to immunize animals with the attenuated strain. After being injected with this attenuated virus their immunity was tested with a virulent strain of heartwater. The result is recorded in the table below:—

TABLE 4.

No. of Animal.	Species.	"Immunised" with Attenuated Strain.	Date.	Result.	"Immunity" Test with Virulent Blood. Date.	Result.
21471	Sheep	98th generation	25.10.29	Doubtful Reaction	20.11.29	Severe Reaction.
22407	,,	,, ,,	,,	,, ,,	,,	No ,,
23601	,,	,, ,,	,,	,, ,,	,,	Severe ,,
23687	,,	,, ,,	,,	Mild ,,	,,	*Death.*
24434	,,	,, ,,	,,	No ,,	,,	,,
24446	,,	,, ,,	,,	Mild ,,	,,	Severe R.
24490	,,	,, ,,	,,	,, ,,	,,	*Death.*
24539	,,	,, ,,	,,	No ,,	,,	,,
24561	,,	,, ,,	,,	Mild ,,	,,	,,
24621	,,	,, ,,	,,	No ,,	,,	Severe R.
24624	,,	,, ,,	,,	Mild ,,	,,	*Death.*
24631	,,	,, ,,	,,	Doubtful ,,	,,	Severe R.
24633	,,	,, ,,	,,	,, ,,	,,	,, ,,
24637	,,	,, ,,	,,	Mild ,,	,,	No ,,
24653	,,	,, ,,	,,	Doubtful ,,	,,	*Death.*
24683	,,	,, ,,	,,	,, ,,	,,	No Reaction.
24693	,,	,, ,,	,,	Good ,,	,,	,, ,,
24714	,,	,, ,,	,,	Mild ,,	,,	,, ,,
23225	,,	102nd ,,	7.1.30	No ,,	10.2.30	*Death.*
23500	,,	,, ,,	,,	Mild ,,	,,	,,
23597	,,	,, ,,	,,	No ,,	,,	,,
24632	,,	,, ,,	,,	,, ,,	,,	,,
416	Cattle	,, ,,	,,	,, ,,	,,	No Reaction.
3508	,,	,, ,,	,,	,, ,,	,,	,, ,,
3520	,,	,, ,,	,,	,, ,,	,,	,, ,,
3513	,,	,, ,,	,,	Mild ,,	,,	*Death.*
3526	,,	,, ,,	,,	Doubtful ,,	,,	No Reaction.
3544	,,	,, ,,	,,	Mild ,,	,,	*Death.*

It seems justified to conclude from these results that the injection of the attenuated virus had conferred no immunity whatsoever on the sheep and cattle. When these animals were tested afterwards with a virulent strain the majority of them reacted severely and nearly half of them died. It is probable that the reactions and the mortality would have been exactly the same if the attenuated virus had not been injected at all previously.

This result in itself would seem to indicate that it is very unlikely that a method of immunization by means of an attenuated strain would ever be successful. A further obstacle in the way of such a

method was brought out by the experiment, namely the difficulty of keeping the strain alive. With the increasing attenuation of the strain the reactions become correspondingly weaker, until towards the end of the experiment the majority of the animals did not react at all. It should further be remembered that the method of maintaining the strain by repeated subinoculations is a very expensive one.

The chief objections against a method of immunization by means of an attenuated strain of heartwater virus, may be summed up as follows:—

(*a*) The uncertain behaviour of the strain (as regards virulence) from one generation to the next,

(*b*) the difficulty of maintaining the strain when once it is attenuated,

(*c*) the cost of such a procedure,

(*d*) the inadequacy of the immunity conferred by such an attenuated strain.

A few further points of interest in connection with the experiment recorded in Table No. 2 may be mentioned here.

At one stage of the experiment it seemed as if there was strong evidence to show that a milder reaction was obtained when the subinoculation was carried out at the end of the temperature reaction, than when it was performed at the beginning or at the height of the reaction. Further evidence was collected on this point, but eventually it was found that the differences were not constant, and reliance could not be placed on this method.

At another stage of the experiment we were puzzled by the very early temperature reactions which appeared in the injected sheep. Ordinarily the incubation period in heartwater is about 10 days, but in these cases the temperature began to rise 3 to 5 days after the injection. The point was never completely cleared up. It is possible that some unknown mild febrile disease was conveyed through several generations in this way; or the disease may have been a mild form of "bluetongue". In the sub-strain which was started in generation 77b (Table 2), these early reactions were particularly noticeable. Rapid subinoculations led to the disappearance of the heartwater virus, and the substrain was discontinued in generation 83b.

SUMMARY AND CONCLUSIONS.

1. Two experiments are described in which an attempt was made to attenuate the virus of heartwater by passage through sheep.

2. In the first experiment the strain was continued for 20 generations, but appeared to be just as virulent at the end as it was at the beginning of the experiment.

3. In the second experiment the subinoculations were continued through more than 100 generations.

4. Considerable variation in virulence was observed during the first 75 generations, but no constant attenuation could be demonstrated.

5. The total mortality in the experiment was 18.5 per cent. During the first 75 generations it was 25.2 per cent., and from the 76th generation to the end of the experiment 4.6 per cent.

6. It would, therefore, appear that there was a marked attenuation from the 76th generation onward.

7. This attenuation was more marked still towards the end of the experiment when (from the 87th generation onward) no further deaths occurred in the experimental animals.

8. There is, of course, no certainty that the strain would not have regained a certain measure of its original virulence, had it been continued longer.

9. This possibility is emphasized by the observation recorded above, that blood of a sheep in the 90th generation, produced fatal reactions in sheep in a separate experiment.

10. The attenuation of the strain towards the end of the experiment reached such a degree that the majority of the injected animals showed very indefinite reactions or no reaction at all.

11. Immunization experiments carried out with this attenuated strain (98th and 102nd generation) failed to produce any immunity in sheep or cattle.

12. Taking further into consideration the difficulty and expense of maintaining an attenuated strain and the possibility of an alteration of its virulence, it seems unlikely that the method investigated here will ever provide a practical means of immunizing cattle or sheep against heartwater.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

The Microscopic Diagnosis of Heartwater: A Preliminary Note on the Value of Intima Smears.

By C. JACKSON, B.Sc., B.V.Sc., Veterinary Research Officer, Onderstepoort.

The discovery by Cowdry (1925) of *Rickettsia ruminantium* provided a method for the laboratory diagnosis of heartwater which was used at this Institute for some time. Later the method fell into disuse for several reasons and subinoculation of blood from the suspected cases became the sole method to be employed as a regular routine and in which reliance was placed on negative findings, provided more than one animal, known to be susceptible to heartwater, had been injected.

It was found that Rickettsia could by no means be demonstrated in every case with the technique in use. This fact led to the constancy of the occurrence in and indeed even the specificity of Rickettsia for heartwater being called into question—this in spite of the careful researches of Cowdry and the rather irrefutable data provided by him.

A further difficulty, and one which cannot be over-estimated in routine diagnosis, was the length of time commonly required for the demonstration of even a single Rickettsia in a series of sections. Further, much time was occasionally wasted in attempting to come to a conclusion about bodies of doubtful appearance.

In this connection it may possibly be of use to mention some of the bodies which it has been found (as the result of a fairly extensive experience in routine diagnosis) may possibly give rise to doubt. These comprise nuclei of lymphocytes lying in the capillaries (this is dealt with below), deposits (including those originating from the stain), and the mast cells of connective tissue. Of the latter it will suffice to make the following remarks.

In sections of organs mast cells are most frequently encountered in the connective tissue forming the adventitia of small blood vessels. In the lung, however, they are not infrequently met with in positions in relation to the capillaries which may give rise to difficulties. With the (Giemsa) stain used their granules most commonly assume a reddish colour, but they may stain blue, a circumstance which may easily give rise to difficulty when it is not remembered that they are in general somewhat less closely packed and more refractile than the granules of Rickettsia, that they are in contradistinction to Rickettsia

arranged on all sides of the nucleus of the cell,* and that they are not separated from the surrounding cytoplasm by a clear unstained ring (halo).

It has recently been found that these disappointing results are largely referable to the overstaining, i.e. insufficient differentiation, of sections. In our experience the granules of Rickettsia in an overstained section appear to run together (due to the deposition of the dye between the granules), giving the appearance of a dense and darkly coloured mass, which may then, of course, be extremely difficult to distinguish from a cell nucleus. By so modifying the strength of the Giemsa solution and the length of time occupied by the staining and the differentiation as to obtain with thin sections cut from embedded material a delicate transparent pinkish-blue appearance on holding the slide against the light, it has been found possible to demonstrate, and usually with the greatest of ease, Rickettsia in suitably selected organs of all animals dying from heartwater.

Prior to this staining improvement it was thought that the fact of many organs submitted having been fixed in formalin might be responsible for the difficulties encountered, especially since Cowdry had been at some pains to use and to recommend special reagents (e.g. Zenker's and Rigaud's fluids). However, comparisons have shown that formalin is in all respects an excellent fixative for this purpose and it has, in our hands, given results equal to if not better than those obtained by the use of Zenker's fluid. At the time this knowledge was very welcome, since the use of special fixatives would certainly be a complication in cases encountered by field officers and more especially by farmers.

In the course of these investigations considerable attention was paid to the distribution of Rickettsia in the tissues and their relative frequency in various organs. The conclusion was reached that the kidney constitutes by far the most convenient organ for investigation, providing for examination as it does capillaries in "concentrated form," i.e. the glomeruli, which saves greatly in the area of the section to be searched. On the other hand, a negative diagnosis should not be based on the absence of demonstrable Rickettsia from the kidney alone. We do, however, make a practice of returning a negative diagnosis when Rickettsia is absent from the brain (cerebellum and cerebrum) as well as from the kidney. In addition, it may be mentioned that the hippocampus appears from a limited number of examinations to equal any other part of the brain in respect of constancy and frequency of Rickettsia. On the other hand, the adrenal is no longer considered worth the trouble of investigation, so rare and so inconstant is Rickettsia in the majority of cases.

In this connection one other point deserves special mention, viz., the occurrence of Rickettsia in the lung. Quite contrary to the findings of Cowdry, Rickettsia was by no means seldom found in the lung, and in some cases was of frequent distribution.

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* This appearance is often not seen unless the nucleus of the cell be accurately focussed, and it may happen that, turning the mechanical stage, the examiner brings into view a cell which contains an apparently solid sphere of granules. On manipulation of the fine adjustment, the nucleus will be seen in the centre of the granules.

Armed with these data, we felt on much more secure ground in regard to our microscopic diagnosis. Nevertheless, grave difficulties still remained, chief among which were the labour and time involved in the preparation and examination of embedded sections of organs and the difficulty of getting laymen to submit from the field a suitable selection of organs (including brain) for diagnosis. It was felt that if these difficulties could be overcome, or rather avoided, considerable progress would have been made towards placing the microscopic diagnosis of heartwater on a basis both practicable and economical.

It is claimed that these ends have been accomplished by means of the method of intima smears devised by us. This consists in the preparation of smears from scrapings of the tunica intima of certain parts of the circulatory system and the examination for Rickettsia in the endothelial cells thereof.

It was some little time before sufficient practice was obtained in order to make good intima smears. However, once it was found how to prepare good smears with regularity, Rickettsia could be demonstrated in practically all cases dying from heartwater. Scrapings are made by dissecting out a convenient length of the vessel selected, opening it longitudinally with a scissors, spreading it flat on a smooth board—serous surface uppermost—and making scrapings with a sharp knife held at an angle to the surface. The material obtained by so shaving the intima collects on the blade, from which it is transferred immediately, in the form of a thick smear, to a clean slide. The drawing of smears with a slide edge as is done with blood, etc., usually results in too sparse a distribution of the cells and thus doubles the labour of the subsequent examination. It is important that the surface of the vessel first be rid of excessive moisture or blood by slight swabbing, otherwise too great a dilution of the cells results. A little practice will be found necessary before the art is acquired, especially by those accustomed to the technique of making blood and organ smears.

Smears are fixed in absolute alcohol and stained lightly with Giemsa.

In smears made by this method it has been found possible to demonstrate Rickettsia in practically all cases of heartwater in sheep. With regard to the disease in bovines, sufficient data have not yet been collected. When the technique of making intima smears has been satisfactorily acquired, it will be found that the search is reduced to a minimum, i.e. a period of from one to fifteen minutes in the majority of cases.

The examination of a large number of intima smears has incidentally thrown interesting light on the morphology of the Rickettsia in the mammalian host, since, as is well known, cytological details show up much more clearly in smear preparations than in sections, where cells and their constituents are crowded together by pressure, and where shrinkage occurs and staining affinities are modified by the treatment involved. The point relating to staining affinities is especially well illustrated: Cowdry saw, in smears made from ticks, pleomorphic Rickettsia composed of two materials staining violet and reddish respectively. Could he have observed similar characteristics in the

mammalian host, he would have been armed with valuable confirmatory evidence for his contention that the Rickettsia seen in *Amblyomma hebraeum* and in the domestic ruminants are stages in the life-cycle of one and the same organism; for it must be remembered, of course, that in the consideration of Rickettsia, and in deciding whether a given body is or is not a Rickettsia, the staining characteristics are among those factors of prime importance. This dual staining affinity, as well as the characteristic pleomorphism, is well illustrated in intima smears in our possession prepared from sheep. Previously *Rickettsia ruminantium* in mammals has been studied only in section preparations, in which the staining is of a uniform dark blue colour and the units appear as coccoid granules, showing great uniformity as compared with those seen in the tick.

A striking feature of Rickettsia in intima smears is the frequency with which ring forms occur. These are comparatively very large, with a distinct space in the centre of the chromatoid ring. This "space" is often seen to be filled with a material staining a very light shade of the same colour assumed by the chromatoid material. This would indicate that the rings are not hollow structures. It was concluded that this substance enclosed in the ring is not identical with the cytoplasmic matrix ("halo," chromophobic substance) referred to above. The latter never assumes a definite shade of colour as does the former. In diameter these rings may be as much as 1.7μ, leaving the halo out of account. Often the ring is not closed and the resulting structure is reminiscent of a horseshoe or of the appearance assumed in sections by the nucleus of a neutrophile (but on a much smaller scale). These bodies are so characteristic that even when, as the result of an artefact, they are met with lying free and singly, they still can be recognised with great certainty. Certain groups of Rickettsiae appear to be composed entirely of such ring forms; in others the ring forms are mixed with coccus-like and other forms. All intermediate stages between the former and pure coccoid aggregations are seen. Of the groups of Rickettsiae, some units assume a pure blue colour, while others are by contrast reddish or reddish violet. Individual units staining two colours (such as may be seen in Rickettsia in the tick) were identified, both in the case of the ring forms, as described above and also in diplococcal forms. It is, however, in the former that the dual staining affinity is perhaps best seen. It has been suggested that the ring forms are to be regarded as an appearance caused by an artefact, but we do not incline to this opinion. The larger Rickettsia granules assume a variety of irregular shapes in addition to the ring forms. Bacillary forms, however, are rare and never distinctly represented.

The "halo" often stands out in contrast to the cytoplasm of the cell, the latter staining pale blue with Giemsa, while the former takes no colour at all. In many cases it can be seen not only to surround the whole group, but also to permeate everywhere between the granules, surrounding each individual unit. This is well seen in Figs 4, 6, and 7, also in Fig. 5, in which, on close examination, it will distinctly be seen that of the two isolated bodies near the top

right-hand edge of the field, the larger and ring-shaped one is provided with its own halo of cytoplasm. Another circumstance throwing further light on the true nature of this halo emerges from a study of those figures (3, 4, 5, 6, and 8) in which clumps of Rickettsiae situated close to the nucleus (a typical position) are exerting pressure thereon, causing the nucleus to adapt itself to the shape of the edge of the clump. In these cases it will often be seen (Figs. 4, 5, and 6) that the " halo " is not obliterated by the pressure. It appears, therefore, that the " halo " is definitely a chromophobic cytoplasm, as was thought by Cowdry.

It was at the outset thought possible that the factors mentioned on page 163 as modifying the appearance of cytological details in sections might indeed account for all the differences between the Rickettsia as seen in organ sections and as seen in intima smears. Further study, however, has forced us to the conclusion that this opinion was scarcely tenable, especially in view of the facts—

(*a*) that in smears the much larger and pleomorphic forms described above did not occur alone, but along with the small coccoid forms which resemble those seen constantly in organ sections; and

(*b*) that while differences in staining might be accounted for by the treatment involved in fixation and embedding, the factors leading to shrinkage could scarcely be so formidable as to cause a reduction in the apparent size of the granules of the larger types to as small a diameter as one of .2–.4μ (the size of the coccoid forms seen in sections); neither would one expect that no evidence whatever of the striking pleomorphism seen in the smears would emerge in the sections.

For such reasons, we must advance the view that in the intima of the larger vessels occurs a form of Rickettsia different from that found in the capillary endothelium. The question as to whether this is possibly a developmental form is at present purely speculative.

In regard to the distribution of Rickettsia in the vascular system, it is difficult to indicate any one portion as being a constant seat of these bodies. In general they are rare in the arterial side as compared with the venous side of the system. The jugular vein and the right side of the heart (especially the atrium) stand, perhaps, first in respect both of constancy and frequency of Rickettsia. Then come the posterior vena cava, left atrium, pulmonary vein, etc. This sequence makes no pretence to exactitude, but is based on general impressions obtained during examinations. Unexpectedly Rickettsia was found to be rare in the renal vein (which drains that organ, perhaps, most suitable of all for the demonstration of Rickettsia in sections). Rickettsia was not often found in intima smears made from arteries, which are, therefore, of litle use in routine diagnosis.

Smears were made from various serous membranes, notably the pericardium, epicardium, and the visceral pleura, since it was considered possible that Rickettsia in its distribution might not be confined to endothelium proper, but might extend to mesothelium also.

The results were rather inconclusive: Rickettsiae were certainly found in such smears, but with such inconstancy and in such small numbers, that the possibility could not be excluded that the cells containing them had been scraped from the underlying capillaries. We incline, indeed, to the opinion that such was the case. The possibility of the occurrence of Rickettsia in the mesothelium is interesting theoretically, especially in view of the characteristic transudation which takes place into the cavities lined by serous membranes, but has no practical application in diagnosis.

It is suggested that, for the present, intima smears submitted from cases of suspected heartwater should be made from at least three or four sites, if absolute reliance is to be placed on negative findings. Present experience dictates that these should be: the external jugular vein (or one of its two radicles), the right atrium (or ventricle), possibly the posterior vena cava, and the left side of the heart.

Summary.

1. *Rickettsia ruminantium* is found with great regularity in intima smears made from sheep dying from heartwater.

2. This affords a rapid method of diagnosis, replacing both the cutting of sections and the subinoculation of blood hitherto in use, and thus accomplishing within the space of minutes what previously required some days.

3. The technique of making intima smears is described.

4. Notes are added on the staining affinities, morphology, and distribution of Rickettsia in both the capillaries and the wider parts of the blood-vascular system.

5. The difficulties previously encountered in the microscopic diagnosis of heartwater are reviewed.

6. The main conclusions of Cowdry (1925) were fully confirmed in these investigations.

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EXPLANATION OF PHOTOMICROGRAPHS.

Intima smears fixed in absolute alcohol and stained with Giemsa. Sections fixed in formalin, embedded, and stained with Giemsa. The magnification is in all cases 1,200 diameters.

FIG. 1.—Two groups of Rickettsiae within an endothelial cell. The granules are purely of the coccoid type. From an intima smear of the jugular vein (Sheep 24711).

FIG. 2.—Another cell from the same preparation as Fig. 1. Of the three groups, that in the middle contains pleomorphic granules of intermediate size, the other two uniform coccoid granules.

FIG. 3.—Two groups of Rickettsiae on opposite sides of the nucleus of an endothelial cell. The smaller has granules of cocroid type; in the larger one the granules are mainly of the ring type. Note the marked pleomorphism. Intima smear from jugular vein (Sheep 26873).

FIG. 4.—Groups of Rickettsiae—pure ring type. Note flattening of nucleus and halo. Smear from the endocardium (Sheep 28903).

FIG. 5.—Rickettsia in an intima smear from the jugular vein. The lowest cell contains a small group of large ring and horseshoe shaped granules. Note flattening of nucleus and halo. In the middle is a group of mixed granules of intermediate size. At the top right are two isolated units (note halo of the larger one), illustrating the certainty with which they may be recognized when lying free (Sheep 24564).

FIG. 6.—Rickettsia in an intima smear from the jugular vein. Halo well shown (Sheep 28950).

FIG. 7.—From a smear of the endocardium. Granules rather scattered and thus showing to great advantage the pleomorphism. Note the chromophobic cytoplasm contrasting with the cytoplasm of the cell (Sheep 24711).

FIG. 8.—Endocardial smear. Small group of large-sized granules causing flattening of the one pole of the nucleus (Sheep 28301).

FIG. 9.—Rickettsia in a smear made from an infected larval *Amblyomma hebraeum*. Note similarity, especially in respect of pleomorphism, between these granules and those in the foregoing figures (Path. No. 11049).

FIG. 10.—Section of hippocampus (10528) rather overstained. Two groups of Rickettsiae in the endothelium of a capillary and between them a lymphocyte. With slightly deeper staining the differentiation may become difficult.

FIGS. 11 AND 12.—Section of lung (Bovine 9507), to illustrate further the difficulties referred to in the text. In Fig. 11 the granules are those of an histogenous mast-cell; in Fig. 12 are several groups of Rickettsiae in the same section.

FIG. 1.

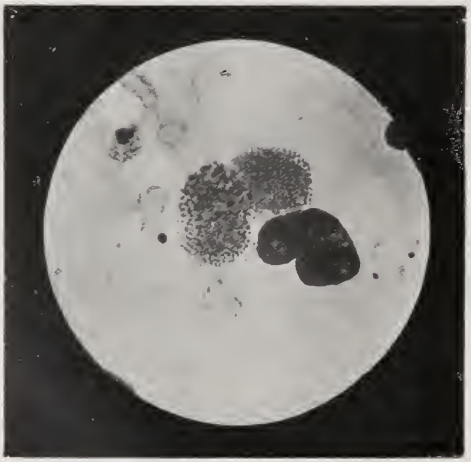

FIG. 2.

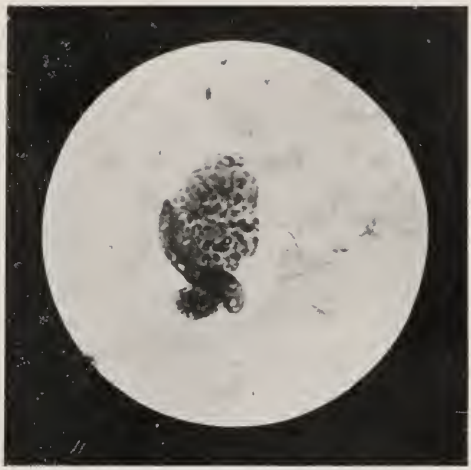

Fig. 3.

Fig. 4.

FIG. 5.

FIG. 6.

Fig. 7.

Fig. 8.

FIG. 9.

FIG. 10.

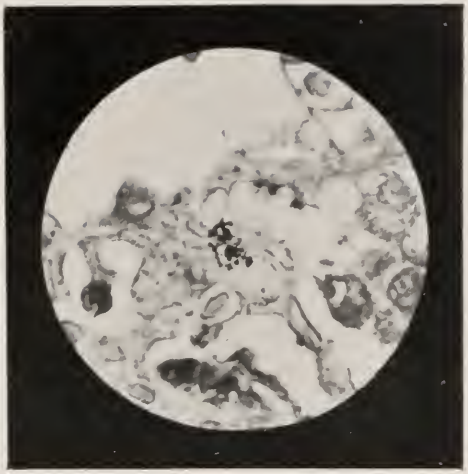

Fig. 11.

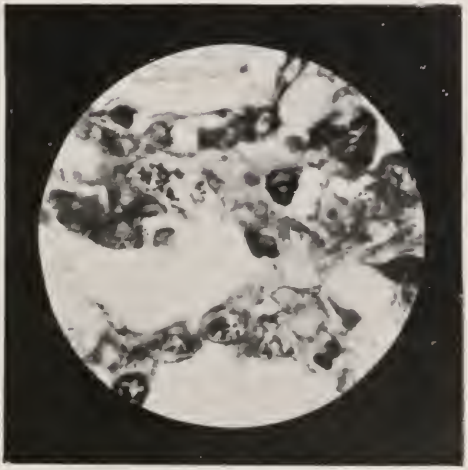

Fig. 12.

A Rickettsia-like Organism in the Conjunctiva of Sheep.

By J. D. W. A. COLES, B.V.Sc., Veterinary Research Officer, Onderstepoort.

During the past two years the writer has studied a disease in sheep in South Africa, generally known as infectious or specific ophthalmia. Clinically, this condition begins as an acute purulent conjunctivitis and frequently, with extraordinary rapidity, develops into an acute keratitis. The eyes remain affected for a period of two to about twenty days. Corneal ulceration, panophthalmia, and subsequent phthisis bulbi occasionally occur. In the great majority of cases the eyes return to an apparently normal state. One eye only is often affected and in such cases the other eye may show symptoms after two or three days. The disease is most prevalent in the spring and summer, and breed, age, sex and locality make no difference. Reports indicate that in some years a quarter of the sheep in South Africa become affected.

It is intended to describe the disease fully in subsequent reports, but a brief summary of what is already known will be given here. The condition appears to be identical with ophthalmia in sheep in Australia and New Zealand. In these two countries and in South Africa eyes of normal sheep have been infected by instilling into the conjunctival sacs the discharges from diseased eyes; the incubation period is two to four days; aerobic and anaerobic cultures (using the usual common media) of organisms in affected eyes did not produce symptoms when instilled into normal eyes, and frequently such media showed no growth at all. Here and in Australia the causal agent has so far been found not to pass through siliceous or plaster of Paris filters. Experiments in this connection are being continued. In Australia it has been noted that the liquid lachrymal secretion loses its virulence in 48 to 72 hours when kept in the ice-chest.

Preparation of Material.

To obtain smears for examination the following technique was employed:—The epithelium lining the inner surface of the upper eyelid was scraped off with a small curette, the lid being held out to prevent pain and injury to the cornea. When the scrapings showed a tinge of blood the material was sprayed over a glass slide and air-dried at once. The smears were fixed by immersion in absolute alcohol for five seconds. They were then air-dried and stained with Grübler's Giemsa (1 drop per 1 c.c. aq. dist. for 1 hour).

These smears often showed the organism about to be described, but not always. At this stage a study was made of the white cells, bearing in mind the relative numbers of leucocytes and lymphocytes in ovine blood. It was soon evident that in the earlier stages of ophthalmia the neutrophiles predominated, whereas they were replaced by lymphocytes when the cornea again became somewhat transparent, thereby indicating that recovery was setting in. Of greater importance, it was seen that the presence of the organisms was very closely associated with the presence of neutrophiles. Only occasionally were they present, and then rarely and usually of the minute blue bipolar rod-shaped intracellular type, when the lymphocytes predominated or when there was no change in the relative numbers of the white cells.

Clinically normal eyes sometimes show the same pictures as diseased eyes when smears are examined. Large numbers of neutrophiles may even be present. The organisms frequently occur in only one eye of an apparently normal sheep. However, it is common knowledge that perfectly healthy animals may show pathogenic organisms such as anaplasms and piroplasms in their bodies, so this fact is not unduly disturbing.

When saline washings of clinically normal eyes containing the organism have been instilled into the conjunctival sacs of new-born lambs (one eye in each case being left as a control), the organisms appeared in smears after three days, some of the lambs showing conjunctivitis as well. The smears of the control eyes remained negative. These experiments are being continued, as the number of cases so far dealt with is too small to permit definite conclusions. It may be mentioned that if the curette is sterile and the operation is performed cleanly, the smears from normal eyes will not vary on subsequent days.

Description of the Organism.

Situation.—It may be extracellular or intracellular (within the conjunctival epithelial cells). Occasionally they are found in neutrophiles, no doubt the result of phagocytosis, but they are then never in clumps.

Morphology.—The organisms are very minute structures. .3 to 1.4 μ by .2 to 1.4 μ, the average being about .8 to 1.0 μ by .5 to .8 μ. Pleomorphism is marked, some being roughly triangular in shape.

The organisms in any particular clump, either extra- or intracellular, are approximately of the one size and inclined to be solid (Figs. 3 and 4) or open (Fig. 5). Fig. 5 shows specimens of the "imperfect ring" type, where the chromatoid material is distinct for about half to three-quarters of the circumference and faint for the rest. It shows also a true horse-shoe form with bulging in of the halo between the arms of chromatoid material. As is well seen in Figs. 3, 4, and 5, the extracellular forms show halos. Sometimes halos are seen round intracellular forms. No capsule could be demonstrated by tinctorial methods.

The open forms (Fig. 5) are very much more commonly found than the solid (Figs. 3 and 4) and their outlines are sharper, although not as distinct as in the case of bacteria.

When the solid forms are well spread out, it is frequently seen that their sides are straight and that they are angular in shape, the impression being that during their development in a clump the individuals had to make the most of limited space. Almost all rod-shaped forms have the bipolar appearance, very few being solid. The smallest forms are mainly rod-shaped; some are coccal, most of these being very minute rings.

The chromatoid material in the larger forms is very dense in spots, which are inclined to bulge (Fig. 5) and give the impression of budding.

Arrangement.—No chains have been seen. The typical picture is a compact mass, either extracellular or intracellular. One mass may fill an entire cell or there may be two or more smaller clumps in the cell. These smaller clumps do not hug the nucleus. Extracellularly the organisms may be found in masses far bigger than any conjunctival epithelial cells, and containing upwards of two thousand individuals. The masses are generally composed of three or four organisms to about five hundred. Masses of the extracellular forms may resemble maize-cobs or rosettes (Fig. 1).

Staining.—Grübler's Giemsa stain has been used almost exclusively, with excellent results. The solid forms take the various shades of purplish-red. The minute bipolar forms stain blue. In the bigger open forms the clear spaces are a very pale blue and the chromatoid material a reddish blue.

The organism is gram negative and not acid-fast. It stains well with fuchsin, eosin, Loeffler's methylene blue, carbolthionine blue, etc., but not as intensely as ordinary bacteria. It stains light purple, supra-vitally, with brilliant cresyl-blue.

Motility.—When stained supravitally and examined at blood-heat no motility was observed. As the organisms were sufficiently numerous, the fresh conjunctival scrapings were mixed with normal sterile saline at 37° C. and examined under a cover slip with reduced light. They were seen, but showed no motility.

Detection.—This is usually very simple in well-prepared smears stained with Giemsa. They have invariably been found in early cases of ophthalmia (over one hundred affected eyes have been examined) and sometimes they are seen in clinically normal eyes as well, but then there is almost certain to be an increase in the number of neutrophiles.

Effect on Tissues.—Before discussing this, it must be admitted that the epithelial cells may be damaged during the preparation of the smears. There does not appear to be any appreciable swelling of infected cells. There does, however, seem to be a point at which the cell membrane ruptures and the organisms are disgorged (Figs. 7 and 9). The cytoplasm of such cells stains more faintly with Giemsa, a probable indication that some form of degeneration has taken place.

Multiplication.—Nothing definite can be said. The peculiar peripheral arrangement of the chromatoid material in the larger ring forms, with the dense specks that bulge, rather suggests the possibility of budding. It cannot be claimed that simple division has been observed. The very large extracellular masses, together with the intracellular (and extracellular) becterium-like organism, which is tion occurs outside the cells as well as within.

The question may now be asked what is this minute, pleomorphic, intracellular (and extracellular) bacterium-like organism, which is gram negative, not acid-fast, non-motile, stains more faintly than ordinary bacteria with the usual dyes, shows red and blue staining with Giemsa, may form several clumps within one cell, and is almost invariably associated with a purulent process. They are not mitochondria which are filamentous and do not appear in compact masses. They are hardly the products of an inflammation, for they will never appear in a perfectly normal eye which has been abused with absolute alcohol, lysol and foreign bodies such as a scalpel. The mere fact that they occur frequently in one eye only would indicate that they are not the products of a systemic disturbance. They cannot be the result of nuclear degeneration for the nuclei of invaded cells are usually intact. They are never present in the eyes of new-born lambs, but may be seen three days after birth. They may at first sight be confused with another intracellular organism, described by the author (1931), but the difference is sufficiently accentuated to allay suspicion.

They are, however, remarkably similar to *Rickettsia ruminantium,* as seen in smears of infected specimens of *Amblyomma hebraeum* (Cowdry, 1926) and in smears of the intima of the jugular and other veins of sheep which have died of heartwater (Jackson, 1931). These preparations were kindly placed at the disposal of the writer by his colleague, Mr. C. Jackson.

From Cowdry (1926), it would appear that this organism does fulfil the requirements, except perhaps one, which would enable it to be classified as a Rickettsia. The exception is the fact that as yet no arthropod vector has been found. Whether this is sufficient reason for invalidating the claim is doubtful.

Up to the present only two Rickettsias associated with sheep have been described—*R. ruminantium* and *R. melophagi.* That this organism is not the former is obvious, as there was never any mortality due to heartwater.

R. melophagi in keds and in cultures made from the blood of sheep shows, according to Nöller (1917) and Nöller and Kuchling (1923), the presence of threads and dumb-bells. These forms are not encountered in the case of the new organism. Moreover, the organism now described spread from sheep to sheep in nature after the animals had been shorn and freed of *Melophagus ovinus* by repeated dipping.

From what has been said it is believed that the organism constitutes a new species of Rickettsia.

Summary.

(1) An organism has been described which is associated constantly with specific or infectious ophthalmia of sheep in South Africa.

(2) This organism is considered to be a Rickettsia, and the name *Rickettsia conjunctivae* is provisionally proposed for it.

Acknowledgments.

The author is deeply indebted to D. A. Gill of the Veterinary Laboratory, Wellington, New Zealand, for a personal communication dealing with the disease in that country, and also to G. Edgar, of the Glenfield Veterinary Laboratory, New South Wales, for a lengthy personal communication embodying the results of his own most interesting work on ophthalmia in sheep in Australia.

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DESCRIPTION OF PHOTOMICROGRAPHS.

All smears were fixed in absolute alcohol for five seconds, air-dried, and stained for 1 hour with Grübler's Giemsa (1 drop per 1 c.c. aq. dist.).

FIG. 1.—1,700 ×. Extracellular rosette clump. Stained reddish with deep purple dots.

FIG. 2.—1,700 ×. Extracellular clump. Reddish with deep purple dots. Margins of individual organisms ill-defined.

FIG. 3.—1,700 ×. Extracellular mass of solid type of organisms staining reddish and deep purple.

FIG. 4.—1,700 ×. Same as Fig. 3.

FIG. 5.—1,700 ×. Scattered mass of open type of organisms showing reddish blue chromatoid material and pale-blue clear areas. "Imperfect ring", horseshoe, triangular, and bipolar forms are present among others more diversely shaped. Halos are distinctly visible, also "budding".

FIG. 6.—1,000 ×. Two infected conjunctival epithelial cells. Note the neutrophiles. Clinically this eye was normal.

FIG. 7.—1,000 ×. Infected and clean cells, side by side. The infected cell has ruptured.

FIG. 8.—1,000 ×. Extracellular clumps of varying size. The cytoplasm of the epithelial cells has broken away disgorging the organisms. The cytoplasm of such damaged cells stains very faintly with Giemsa.

FIG. 9.—1,000 ×. Normal and infected epithelial cells, the latter breaking up. Several extracellular masses, one very large, are seen.

Fig. 1.

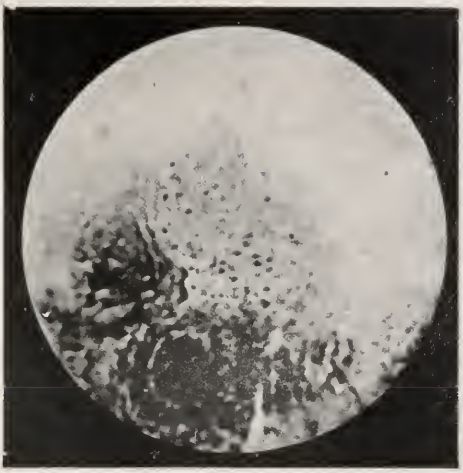


Fig. 2.

Fig. 3.

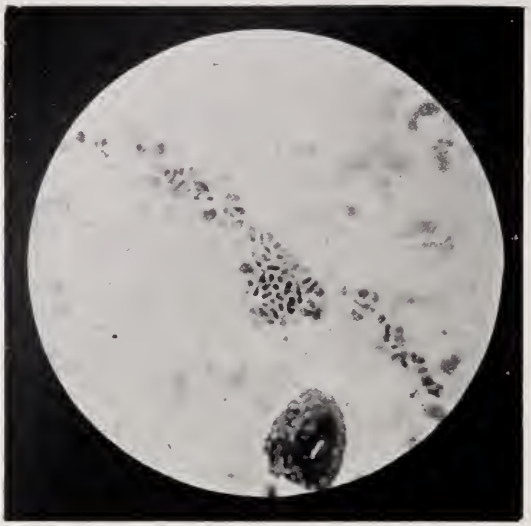


Fig. 4.

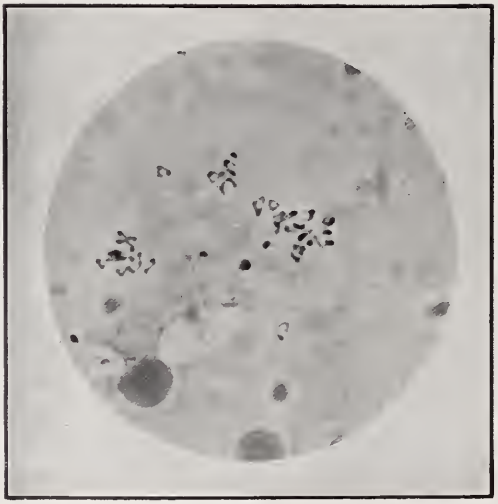


Fig. 5.

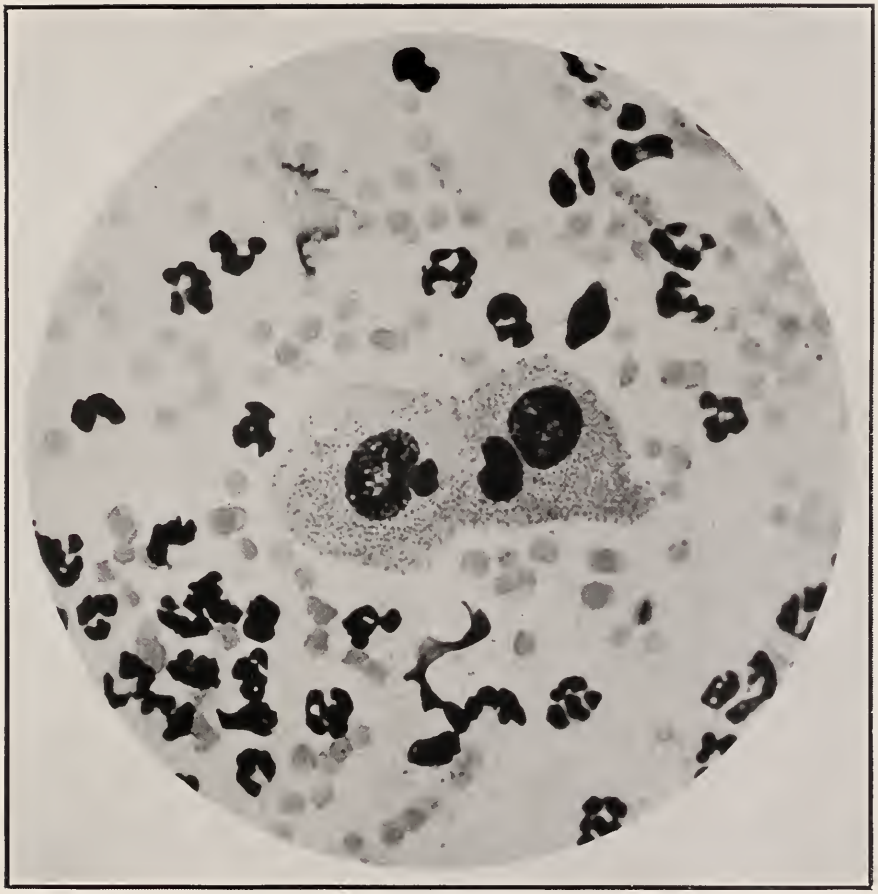


Fig. 6.

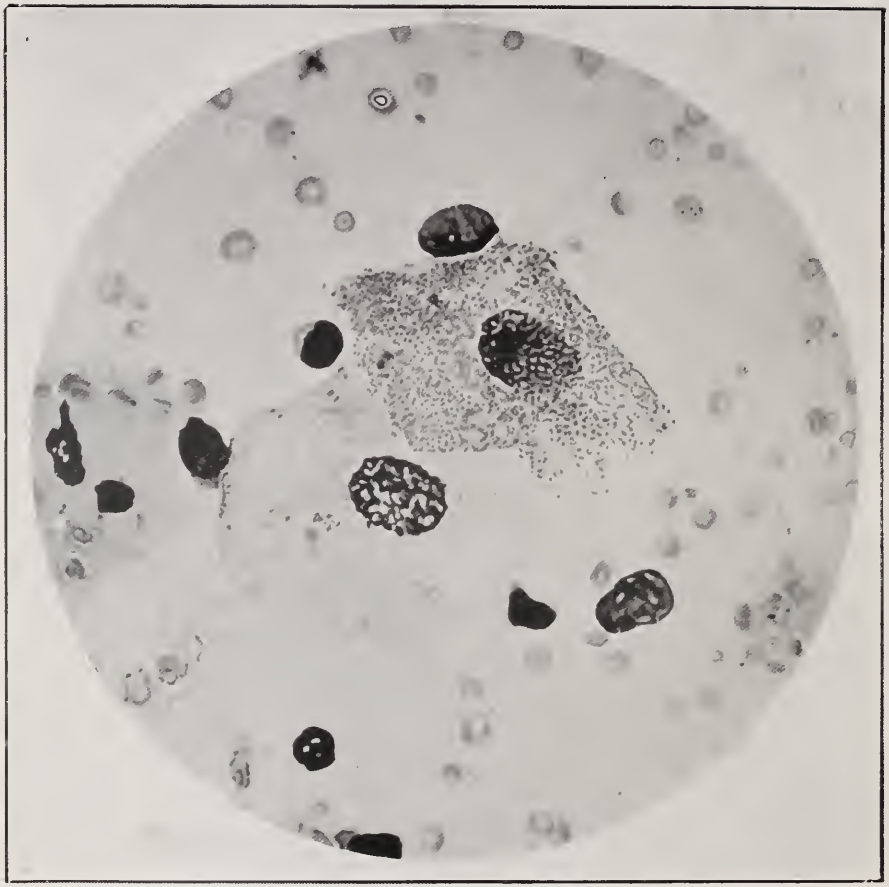


Fig. 7.

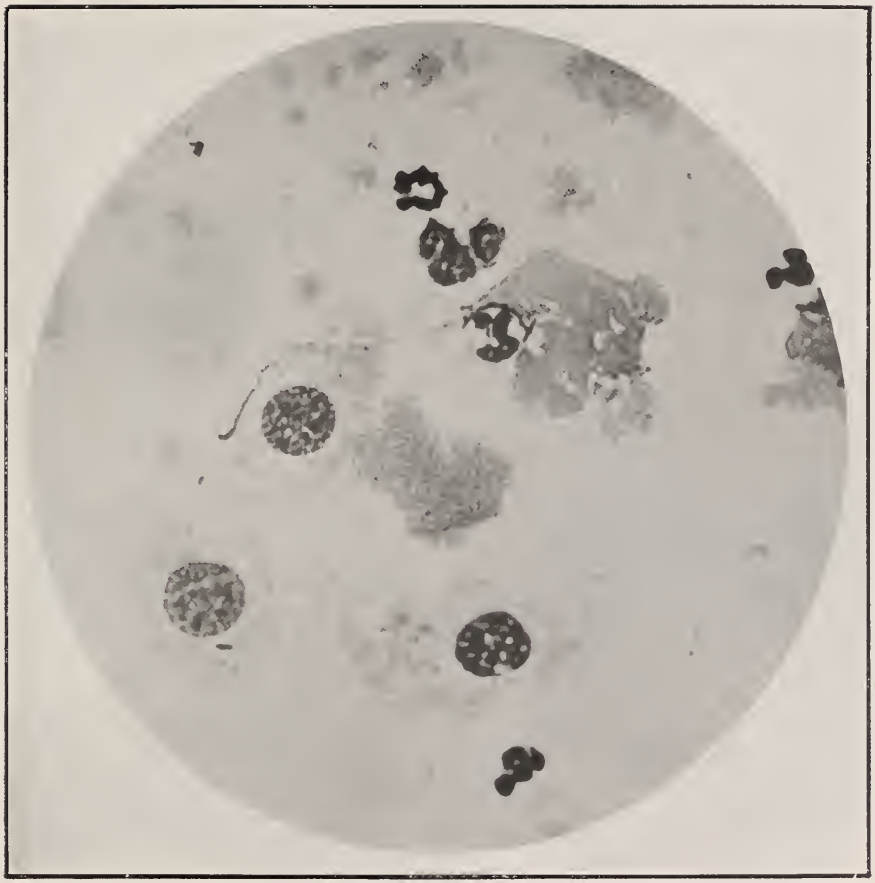


Fig. 8.

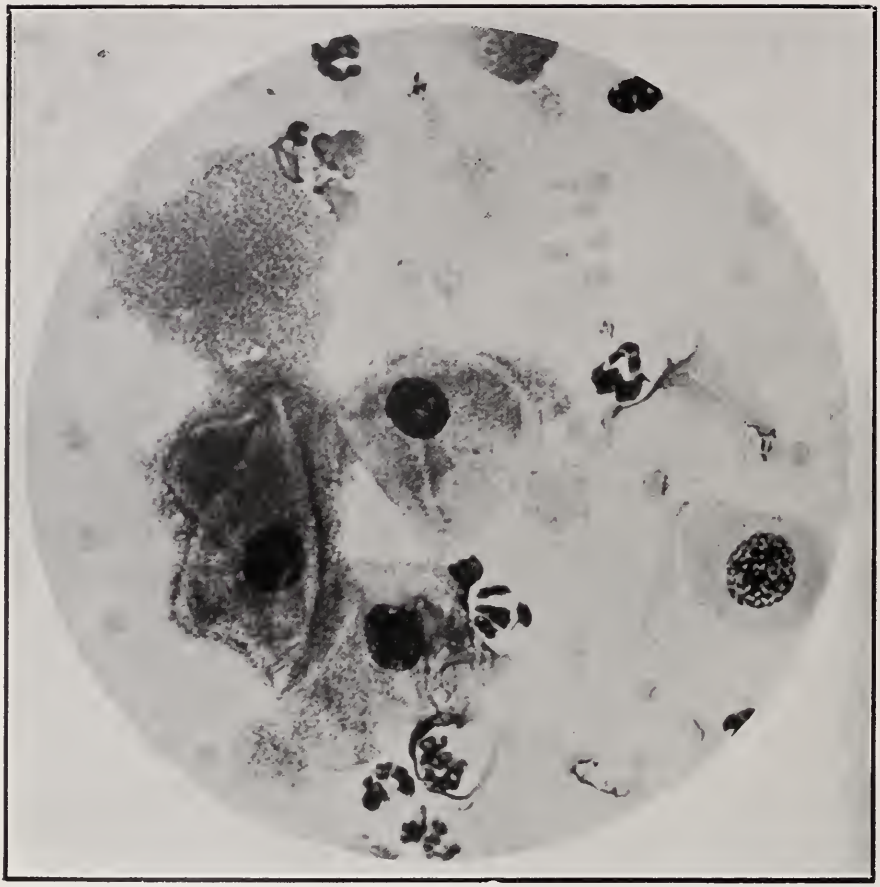


Fig. 9.

An Unknown Intracellular Organism of the Conjunctival Epithelium of Sheep. Preliminary Report.

By J. D. W. A. COLES, B.V.Sc., Veterinary Research Officer, Onderstepoort.

In a paper on *Rickettsia conjunctivae* by the author (1931) reference was made to an intracellular organism of the conjunctival epithelium, which might possibly be confused with the Rickettsia.

It has been observed in the eyes of thirty odd sheep, and on three occasions in connection with *R. conjunctivae*.

Smears for examination were prepared according to the method mentioned in the above article.

Description of the Organism.

Situation.—It is almost exclusively intracellular when the number is small, but extracellular forms are by no means rare when the infection is heavy. When intracellular, it has been seen only in epithelial cells. It may be present in one eye only.

Morphology.—The typical organism is rounded but slightly elongated and even slightly comma-shaped individuals are encountered. Pleomorphism is not very pronounced. In size it is .5 to 1.8 μ by .3 to 1.6 μ, the average being about 1.2 by 1.0 μ.

On the whole it is thus larger than *R. conjunctivae*. The deeply staining material is arranged usually more or less in the form of a quarter to half moon, the rest of the organism staining very faintly indeed. This dark-stained substance is relatively more prominent than in the case of *R. conjunctivae*.

Halos appear round both the intra- and extra-cellular organisms. No capsule has been revealed by staining.

Disposition.—Unlike *R. conjunctivae*, clumping is not a feature of this organism's arrangement. The separate elements are dispersed more or less evenly throughout the cell cytoplasm, and the extracellular forms show no tendency towards mass formation. It is unusual to find more than sixty organisms in any one cell (Fig. 2).

Staining.—Like *R. conjunctivae* it is gram negative, not acid-fast, and stains a little less intensely than ordinary bacteria with the usual dyes. Unlike *R. conjunctivae*, it shows no red with Giemsa, but the half-moon part stains deep blue and the rest a very faint blue.

Multiplication.—Below and to the right of the nucleus in Fig. 2 a sort of dumb-bell form may be seen. Such forms are not uncommon and suggest the possibility of simple division.

Detection.—This is very simple, especially in smears stained with Grübler's Giemsa.

Cultivation.—The results have so far been negative, using ordinary common media.

Effect on Tissues.—Sheep harbouring these organisms exhibit no conjunctival or corneal lesions. The smears reveal no disturbance in the absolute and differential white cell counts. The cytoplasm of affected cells stains normally with Giemsa. It would appear that the extracellular forms result from the normal casting off and subsequent breaking up of the superficial epithelial cells. In other words this organism is at present regarded as a symbiont.

Discussion.

A search through the literature reveals no mention of such an intracellular organism in mammals. As this is merely a preliminary report intended to prevent confusion with *R. conjunctivae*, and as further investigation is essential, it is not necessary at this stage to offer a comparison with the intracellular symbionts of certain invertebrates.

Summary.

An intracellular, gram-negative, non acid-fast and apparently harmless organism of the conjunctival epithelium of sheep has been described. Its exact nature is at present unknown.

REFERENCE.

COLES, J. D. W. A. (1931). A Rickettsia-like Organism in the Conjunctiva of Sheep. See this Report.

DESCRIPTION OF PHOTOMICROGRAPHS.

The smears were fixed in absolute alcohol for five seconds, air-dried, and stained with Grubler's Giemsa (1 drop per 1 c.c. aq. dist. for 1 hour).

Fig. 1. —850 ×. Conjunctival epithelial cells of a sheep infected with the organism. Note the dispersed arrangement and lack of clumping.

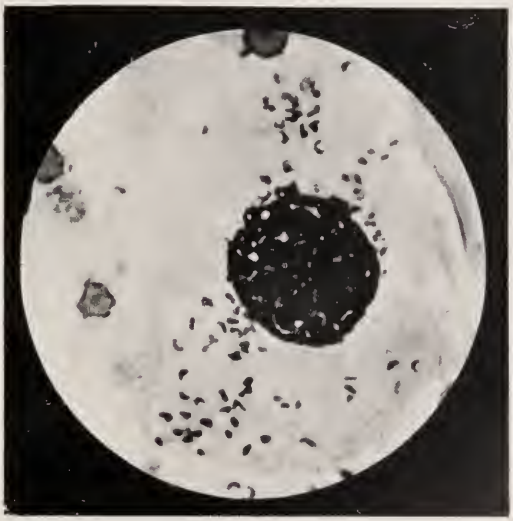


Fig. 2.—1,700 ×. One infected epithelial cell. Note the halos and the somewhat dumb-bell-shaped individual below and to the right of the nucleus.

Section III.

Bacteriology and Fungus Diseases.

J. Scheuber ... ... Black Quarter and Malignant Oedema. A New Method for the Preparation of a Bi-valent Vaccine against *B. chauvaei* and *Vibrion septique*.

P. S. Snyman ... *Vibrio foetus* as a Cause of Bovine Abortion in South Africa.

D. G. Steyn ... ... Investigations into the Cause and Transmission of Lumpy Wool affecting Merino Sheep and its Treatment.

A. D. Thomas ... Actinobacillosis and other Complications in Sheep which may arise from the Feeding of Prickly Pear (*Opuntia* spp.).

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Black Quarter and Malignant Oedema.

A New Method for the Preparation of a Bi-valent Vaccine against *B. chauvaei* and *Vibrion septique*.

By J. R. SCHEUBER, Dr.Med.Vet., Research Officer, Onderstepoort.

The indication for the use of a bi-valent vaccine against black quarter and *Vibrion septique* is evident whenever doubts may exist in regard to the etiology of certain outbreaks of so-called black quarter.

What is generally termed " typical black quarter lesions " does not allow of the conclusion that there was an etiological association with *B. chauvaei*. On the other hand, a marked haemorrhagic oedema in the subcutaneous tissues, together with a comparative or complete absence of dark, spongy muscles, as is often found in sheep dead after shearing, can by no means be taken as an indication of a *Vibrion septique* infection; all such cases examined by the writer proved to be genuine black quarter.

Bacteriological examination may not dispel all uncertainty in cases where several pathogenic organisms are found to be present in the specimen submitted. Occasionally *B. chauvaei* is masked and rapidly outgrown by *V. septique* and a wrong diagnosis may be the result.

If, therefore, the slightest doubt exists, a bi-valent vaccine is preferable to the simple one. Suggestions, experimental data, and practical results in the field have been published by different research workers.

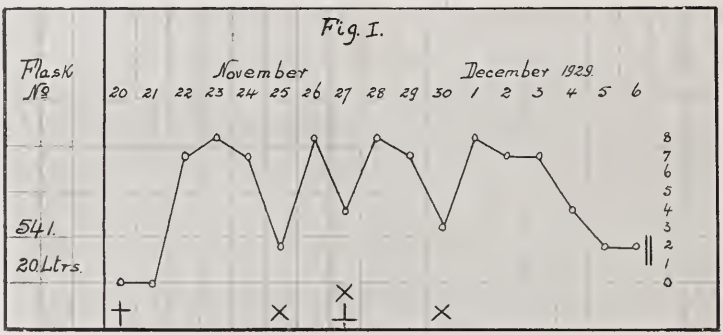
Uchimura (1921) suggested a mixture of filtrates prepared from black quarter, malignant oedema, and *B. putrificus* cultures. Maeninger (1924) reported good results obtained with a mixture of black quarter and *V. septique* filtrate. Viljoen and Scheuber (1926) published experimental evidence to the same effect.

Allen and Bosworth (1924) protected guinea-pigs against both organisms by a combined injection of *V. septique* toxin-antitoxin mixture and black quarter filtrate. Dalling, Mason and Allen (1926) found their vaccine consisting of underneutralized *V. septique* toxin-antitoxin and black quarter filtrate effective against braxy.

The method described below, allowing the preparation of a bivalent vaccine by growing both germs in one flask is based on the technique described by Viljoen and Scheuber (1926). The culture medium consisting of one part of minced liver boiled with two parts of distilled water, and containing Peptone 1 per cent., Sodium chloride .3 per cent, and Sodium phosphate .2 per cent., is filled into flasks of 20 litre capacity and sterilized at 120° C. for five hours on two successive days. Then 1 per cent. of glucose, sterilized twice for one hour at 105° C. in concentrated solution, is added.

Each flask is seeded with 20 c.c. of virulent *B. chauvaei* culture, incubated at 38° C. and allowed to grow for one week. A satisfactory growth is maintained by the addition of 1 per cent. (or $\frac{1}{2}$ per cent.) of glucose and sufficient sodium hydroxide to bring the medium to about P.H. 8, whenever the growth, as judged by the development of gas, becomes rather weak.

After one week 20 c.c. of *V. septique* culture are added and a good growth is maintained for another 8-10 days. The culture is then tested for purity. Figure 1 gives the inoculations, growth curve and additions of glucose and sodium hydroxide for flask No. 541.



Explanation.

Growth curve: 0. No growth.
1. A few gas bubbles.
2. Up to $\frac{1}{4}$ of surface covered with gas bubbles.
3. $\frac{1}{4}$ to $\frac{1}{2}$ of surface covered with gas bubbles.
4. $\frac{1}{2}$ to $\frac{3}{4}$ of surface covered with gas bubbles.
5. $\frac{3}{4}$ to whole of surface covered with gas bubbles.
6. Layer of gas bubbles up to $\frac{1}{2}$ in. thick.
7. Layer of gas bubbles up to $\frac{1}{2}$–$1\frac{1}{2}$ in. thick.
8. Layer of gas bubbles over $1\frac{1}{2}$ in. thick.

† Injection with 20 c.c. of *B. chauvaei* culture.
× Addition of glucose and sodium hydroxide.
$\overset{\times}{+}$ Injection with 20 c.c. of *V. septique* culture.
‖ Removed from incubator. Purity test.

0.4 per cent. of formalin (=40 per cent. solution of formaldehyde) is added, the culture is shaken and then left standing for 24 hours in order to allow the coarser particles to settle. The supernatant fluid is then drawn off, incubated for at least one week and then tested for sterility by subculturing sufficiently large quantities (at least 50 c.c. from each flask) into brain medium. If no evidence of growth is discovered during 3-4 days, the vaccine is tested for safety and immunizing properties by inoculation into sheep. The vaccine prepared from flask 541, mentioned above, was injected into four sheep, each receiving 5 c.c. subcutaneously into the thigh. The vaccine produced neither swelling nor rise of temperature. Three sheep showed slight and the fourth one moderate lameness the day following the inoculation, but 24 hours later this had entirely disappeared again.

The result of the immunity test can be seen from Table 1.

Table 1.

Sheep Nos.	Bivalent Vaccine 18/12/29.	Reaction.	Vir. B.Q. cult. 7/1/30.	Result.	Vir.V.sept. culture 14/1/30.	Result.
21909	5 c.c. subcut.	Moderate	2 c.c. intramusc..	Lived	0·5 c.c. intramusc.	Lived.
22573	,,	Slight	1·5 c.c. ,, ..	,,	,, ,,	,,
23206	,,	,,	2 c.c. ,, ..	,,	,, ,,	,,
23247	,,	,,	1·5 c.c. ,, ..	,,	,, ,,	,,
24421	CONTROLS.		2 c.c. intramusc..	Died 10/1/30		
24478			1·5 c.c. intramusc.	Lived.		
20885	CONTROLS.				·5 c.c. intramusc.	Died 15/1/30
24538					,, ,,	Died 15/1/30

As the control sheep receiving 1.5 c.c. of *B. chauvaci* culture survived, the conclusion in regard to immunity against black quarter can only be drawn from two sheep, but appears to be satisfactory. The immunity against *V. septique* was good, both control sheep dying the day following the injection.

Conclusion.

By superinoculating a *B. chauvaci* culture with *Vibrion septique* and maintaining a good growth by adding glucose and sodium hydroxide whenever necessary, a formalized culture vaccine can be obtained which protects against both diseases.

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17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Vibrio foetus as a Cause of Bovine Abortion in South Africa.

By P. S. SNYMAN, B.V.Sc., Veterinary Research Officer, Onderstepoort.

The purpose of this article is to record that abortion in cattle associated with *Vibrio foetus* has been encountered, and that the organism has been successfully cultivated on artificial media in South Africa.

Yakimoff on the question of Spirillae in bovine abortion seems of opinion that *Vibrio foetus* should be considered alongside *Br. abortus* as a cause of abortion. McFadyean and Stockman were the first to investigate abortion due to Spirillae in sheep and also encountered the organism associated with abortion in cattle in Ireland and Wales. Smith in America observed some fourteen cases of abortion from which pure cultures of the Vibrio were obtained. Subsequently various workers have described Vibrio abortion both in sheep and cattle in America and Europe.

Up to the present, abortion associated with a Vibrio was not encountered in this country until recently in a dairy herd near Pretoria, abortions of an epizootic nature took place, and all the sera of the aborting cows were negative to the routine agglutination test for contagious abortion (Bang's disease). Another cause for the abortion was therefore sought.

The history of the herd in which the abortions took place is shortly as follows: The herd consists of some 160 cows. No new dairy stock except bulls was introduced into the herd for the last 20 years, and no abortion has ever occurred. A year before the abortions started a flock of 200 sheep were brought on to the farm, and these sheep are also the only sheep on the farm. No abortions have taken place in the flock of sheep to the knowledge of the owner.

In August last year one cow aborted, which was also the first known case since the foundation of the herd in 1910. By December 8th abortions had taken place, when sera of the aborting cows were first subjected to the agglutination test for Bang's disease with negative results. However abortions continued and arrangements were made for the examination of a foetus as fresh as possible. In January of this year a foetus dropped three hours previously was examined. On autopsy all conditions described by Smith were present. These conditions have also been observed in foetuses dropped in Brucella infection, viz., oedema of subcutaneous tissues with effusions into the larger serous cavities, the stomach contents consisting of a thick, turbid, yellowish fluid.

Coverslip preparations of stomach contents were microscopically examined and Spirrilae, both long and short, active moving forms, were seen. Smears stained with Carbol Thionin also showed both long and short forms. (See Figures 1 and 2).

To tubes containing serum agar and to which serum broth and defibrinated horse blood had been added, about .5 c.c. of stomach contents were added. Some tubes were sealed with paraffin wax and incubated at 37° C., while others were placed in screw-top fruit jars with soldered tops. The top was connected with a y-piece to a barometer and a suction pump. The air was withdrawn until mercury dropped one-third of its original height, and CO_2 let in from a container until the original column of mercury was restored. The top was then closed and the jar placed in an incubator at 37° C.

After five days, growth was visible in both sets of tubes. The sealed ones showed a faint growth at the junction of the solid and fluid media, and an undulating greyish zone in the capillary space between the solid media and tube, similar to that described by Smith. The tubes in the fruit-jar, on theother hand, showed a more profuse growth, especially in those to which horse blood had been added. The growth in these tubes extended for about 1 cm. along the solid media in a greyish film, while the serum was slightly cloudy. Stained smears (Carbol Thionin for 5 minutes), Figure 3 revealed that a pure culture of the organism had been obtained. Subcultures are now in the 9th generation and the fruit-jar method of cultivation has been adopted as most convenient and satisfactory. Cultures also now grow, i.e. 10th generation, in air, but not so well as in fruit-jars.

Cultural Characters of Organism Isolated.

On plain serum agar the surface growth appears in small colonies, like tiny honey drops. Where seeding has been thick, greyish confluent growths appear, and held up to the light they are very refractile, not unlike *B. abortus*. The morphological characters also correspond very closely to those described by Smith, and Smith and Taylor, i.e. hanging drop preparations of stomach contents and of cultures the Vibrio show a thin wavy, rapidly moving organism. In the first eight generations both long and short forms were present, with the proportion of long forms becoming less as subsultures were made. Long forms up to 30 waves have been seen, while short forms appear as bent rods, or V-shaped with an obtuse apex.

In smears of the 5th generation stained with a watery solution of Giemsa (two drops stain to 3 c.c. water for two hours) two distinct types were observed. The common type stained purplish like the original organisms in the stomach contents and a bluish thinner form in varying lengths up to 8 waves was observed. The first are the capsulated and the latter the naked forms.

Granules are also very distinct in old cultures, appearing like cocci at irregular intervals along the wavy line in both long and short forms.

The Relation of the Organism Isolated to the Cases of Abortion in the Herd.

In all the thirteen cases of abortion the sera of the cows were subjected to the agglutination test. Cultures of the organism isolated were washed off in carbol saline and the emulsion was well shaken to break up any clumps that might exist. As control a serum giving a positive agglutination reaction to *Br. abortus* was used.

The table given below gives a short history of the cows with the result of the agglutination reaction.

Name of Cow.	No. of Normal Calves.	Date Served.	Date Aborted.	Agglutination Test.							Remarks.
				1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	
Lilian	8	7/6/30	12/11/30	++	+	−	−	−	−	−	All the cows calved regularly every season except Bontrok, which had two regular calvings, missed one season and two consecutive normal calves immediately before the abortion.
Bontrok	4	3/8/30	7/11/30	++	++	−	−	−	−	−	
Dina	3	20/8/30	9/11/30	++	++	++	−	−	−	−	
Molly	4	22/7/30	8/10/30	++	++	++	+	−	−	−	
Daisy	1	22/7/30	17/10/30	++	++	++	+	−	−	−	
Soetmuis	4	6/4/30	15/9/30	++	+	−	−	−	−	−	
Clara	6	−/5/30	16/10/30	++	++	−	−	−	−	−	
Violet	6	?	14/10/30	++	++	−	−	−	−	−	Controls tubes: Vibrios in saline remained in suspension.
Effie	3	?	1/12/30	++	++	+	−	−	−	−	
Jes	6	−/7/30	8/12/30	++	++	+	−	−	−	−	
Sanna	4	−/10/30	15/12/30	++	++	+	−	−	−	−	
Iris	5	10/8/30	14/1/30	++	+	−	−	−	−	−	
Fanny	5	25/7/30	24/1/30	++	++	++	++	+	−	−	
Control serum No. 3507 known to give a positive reaction to *B. abort.*				−	−	−	−	−	−	−	

NOTE.—1/10, 1/20, etc. gives ultimate dilutions of serum.
++ complete agglutination.
+ partial agglutination.
− no agglutination.

It may also be mentioned here that about 2 c.c. of stomach contents of the foetus was placed in 15 c.c. sterile saline and left undisturbed in a dark cupboard at room temperature. After 14 days a thick sediment of debris had collected at the bottom. Immediately above this was an almost watery clear zone in the liquid for about 1 cm., then above this zone the fluid became more and more cloudy towards the top, except for the last 2.5 cm. Smears made of a drop of the cloudy saline showed only vibrios, both long and short forms, and coverslip preparations showed them to be very active. The top cloudy saline was pipetted off and well shaken, filtered through blotting paper and used as antigen for an agglutination test with sera of the three last-mentioned cows with the same result as given in the table.

The cases of cows " Soetmuis " and " Molly " are of some interest and, therefore, a fuller history is given in the light of the experience of Smith and co-workers, that vibrios have not been encountered in heifers of the herd calving for the first time. Three cases, however, have been studied by Smith and were considered of sufficient importance for the full history to be given.

Soetmuis' last normal calving was on 2.4.1929 and then she ran with the milk herd until 2.3.1930. At the end of her lactation period she was then placed with the dry stock, consisting mainly of heifers, in a separate paddock and was served in this paddock in April and aborted there on 15.9.30. Molly, with a somewhat similar history, had her last normal calf on 27.2.1929 and was removed to the paddock with the dry stock on 2.3.1930, served there in July and aborted on 8.10.1930.

The question now arises whether the infection was picked up by the two cows while still running with the milk herd before they were served, or does this support the earlier findings of Smith seeing that none of the heifers aborted. Or are heifers more resistant?

It is also probable that Smith's explanation of the case may be accepted here, i.e. that the dry stock were not under such close supervision as the milking cows, but according to the owner the dry stock were inspected daily, and it is very unlikely that any abortions could have been missed.

Small Animal Inoculation.—A male and a non-pregnant female guinea-pig inoculated with stomach contents showed no signs of Brucelli infection and their sera were negative to the agglutination test with *B. abortus*. Four pregnant guinea-pigs and four pregnant rabbits were injected, two of each with vibrio culture and cloudy supernatant fluid from stomach contents and saline mentioned above, the guinea-pigs intraperitoneally and the rabbits intravenously. One of the guinea-pigs injected with cloudy saline aborted during the third night after injection. Cultures from the stomach of the foetus yielded vibrios plus contamination with cocci. Pure cultures were obtained from the uterus of the mother guinea-pig, which was killed in the morning.

General Summary.

Comparing the general characteristics of the organism isolated from the foetus dropped by Fanny with the description given by Smith and his co-workers of *Vibrio foetus*, it is evident that a strain of the organism has been encountered.

That Fanny aborted as a result of *Vibrio foetus* infection is evident, in that a pure culture of the organism was obtained from the stomach of the foetus and her serum gave a positive agglutination reaction to the vibrio and a negative one with *B. abortus*.

That the other 12 cows aborted from the same cause may be assumed because of the positive agglutination reactions of their sera to *Vibrio foetus* and their negative results with *B. abortus*. The organism isolated gave a negative agglutination reaction with a known positive serum for *B. abortus* as well.

From the foregoing it may be definitely concluded that abortion in cattle due to *Vibrio foetus* does occur in South Africa, and according to the summary of the literature by Yakimoff the infection is more widely spread and causes more abortion than is generally accepted. The reason for this is that many cases of abortion due to *Vibrio foetus* remain undiagnosed owing to the difficulty of cultivating the organism artificially and of obtaining foetuses fresh enough for bacteriological examination.

The manner in which the disease was introduced into this particular herd is open to speculation. It is, however, not unreasonable to assume that the infection was introduced with the sheep, seeing that these were the only animals subject to Vibrionic abortion, brought on to the farm, although it has not yet been diagnosed in the latter animals in South Africa.

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FIG. 1.—Magnified×1250.

FIG. 2.—Magnified×1250.

Fig. 3.—Magnified×1250.

Investigations into the Cause and Transmission of Lumpy Wool affecting Merino Sheep and its Treatment.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

In 1925 this disease was brought to the notice of the Veterinary Department and subsequently a large number of cases have been reported from all parts of the Union, especially from those with a heavy rainfall.

This disease is of considerable economic importance, as the wool is damaged and unsaleable and bad cases of the disease end fatally. In advanced cases there is a pronounced loss in condition.

Bekker (1928) was the first to investigate and describe the disease in South Africa, and he suspected "steekgras", excessive dipping, and certain dip preparations of playing a part as predisposing factors. He failed to establish the cause of the disease and was unable to reproduce it artificially.

Bull (1929) described a skin disease, dermatomycosis (lumpy or matted wool) in Australia, the lesions of which are identical with those encountered in lumpy wool in South Africa. He has established the cause of the Australian lumpy wool to be a fungus which he termed *Actinomyces dermatonomus*. The disease affects only young Merino sheep. It is of rare occurrence, but in some flocks he has found up to 20 per cent. of the young animals affected.

Bull was successful in transmitting the disease from affected to susceptible animals both by means of pure cultures of the responsible fungus and by scrapings made from the lesions and applying these to the skin of healthy sheep.

He has furthermore conducted *in vivo* and *in vitro* tests with various antiseptics, and has found that copper sulphate inhibits the growth of the fungus. As a preventive measure, he suggests the dipping of young animals in a 1:500 copper sulphate solution.

Marais (1930), of this Division, has been able to establish the following points concerning lumpy wool:—

(1) In contact experiments proved negative.

(2) Skin of susceptible sheep scarified and scrapings from lumpy wool lesions strewn on the wool over the scarified surfaces gave negative results.

(3) Lumpy wool scrapings rubbed on the scarified spots produced a light transitory reaction.

(4) Sheep treated as in (3) and wool kept moist produced lesions resembling those of lumpy wool.

(5) Young sheep are more susceptible than full-grown ones.

(6) $2\frac{1}{2}$ Per cent. carbolic acid solution prevented the appearance of lumpy wool lesions.

(7) He was able to demonstrate in the lesions the presence of hyphae of mycelium of a fungus, but failed to produce any reaction on the skin of susceptible sheep with cultures made from this fungus.

Incidence.

The great majority of cases of lumpy wool have been reported from areas with a high rainfall and where mists are common. The disease is most prevalent in Natal and the Eastern Cape Province, while it is rarely encountered in the Karroo, and then only as odd cases.

Morbidity and Mortality.

The percentage of animals affected is very low, but the disease seems to be spreading slowly. Moist and warm weather are most decidedly very important factors in determining the morbidity and mortality. The mortality is low, but affected cases, if not treated in time, will end fatally, provided the climatic conditions are favourable for the spread of the disease.

Symptomatology.

The majority of cases of lumpy wool in its early stages of development are detected at the time of shearing, when the "lumps" are found in the fleece.

The disease appears in isolated spots on the back and sides of the affected animal. There is exudation of a yellowish material which dries, and in the course of time a hard conical, and later a pyramidal mass, which mats the wool fibres at that point, is formed. At a certain stage these pyramids become hollow, and these hollow pyramidal masses form the most characteristic symptom of the disease; hence the appropriate term "lumpy wool".

As the disease progresses, these isolated lumps become confluent, with the result that in advanced cases large areas of the skin are covered by hard masses of matted wool (fig. 1).

Fig. 1.—Sheep 29146, showing advanced state of lumpy wool all over the body.

Fig. 2.—Sheep 29146, showing lumpy wool lesions on nose.

Fig. 3.—Sheep 29146, showing lumpy wool lesions on ear.

Fig. 4.—Sheep 29146, showing lumpy wool lesions on leg.

In the worst cases even the ears, nose, and legs are affected (see figs. 2, 3, and 4). It might be mentioned here that the animal referred to in fig. 1, which had been sent to Onderstepoort for experimental purposes, died from the effects of lumpy wool on the sixth day after arrival. It will be referred to later on.

Old lumps can be easily removed, leaving a red granulating surface, whereas those of recent development can be pulled off only with great difficulty and leave a "raw-looking" bleeding surface.

These hard lumps consist of hypo-keratinized epithelium layers mixed with an exudate mainly of the nature of neutrophiles.

In very advanced cases (fig. 1), the old "lumps" are shed and new ones are formed in their place. Only a few small patches of this animal's skin were free from lesions. Movement caused pain, with the result that the animal walked with a stiff gait. It was in a very bad condition, showed anaemia and diarrhoea, and fed very little.

It died on the sixth day after arrival and revealed the following post-mortem appearances: Typical lumpy wool lesions affecting about 80 per cent. of the skin's surface; cachexia; anaemia; chronic bronchopneumonia; *Dictyocaulus filaria* and *Cysticercus tenuicollis*.

The following experiments were conducted at Onderstepoort:—

EXPERIMENT I.

Curative Treatment.

Sheep 29146.—Received from the Government Veterinary Officer, Grahamstown, on 8.9.30. This animal, as stated before, was in the last stages of the disease.

Seven patches (10 cm. in diameter) of the affected skin were selected for this experiment. As it was obvious that any method of treatment would be useless unless the hard masses of matted wool be first removed, five of these patches were soaked with raw linseed oil. After twenty-four hours the lumps were removed without any difficulty and apparently without pain, leaving "raw" granulating surfaces.

The following fluids were then applied to these surfaces:—

(*a*) 1 Per cent. copper sulphate solution.

(*b*) 5 Per cent. copper sulphate solution.

(*c*) 1 Per cent. salicylic acid solution in 96 per cent. alcohol.

(*d*) 3 Per cent. salicylic acid solution in 96 per cent. alcohol.

(*e*) $3\frac{1}{2}$ Per cent. tincture of iodine.

Results.

(*a*) 1 *Per Cent. Copper Sulphate Solution.*—This solution was applied once daily for a period of five days. There was no improvement, the treated patch forming hard crusts, which again developed into the typical lumpy wool.

(*b*) 5 *Per Cent. Copper Sulphate Solution.*—Treated as above with identical results.

(*c*) 1 *Per Cent. Salicylic Acid in* 96 *Per Cent Alcohol.*—Treated once daily on five successive days. The 96 per cent. alcohol had a desiccating effect on the "raw" surface, consequently extremely hard crusts were formed and there was no sign of improvement.

(*d*) 3 *Per Cent. Salicylic Acid in* 96 *Per Cent. Alcohol.*—Treated as in (*c*) with identical results.

(*e*) 3½ *Per Cent. Tincture of Iodine.*—Also in this case the alcohol caused hardening of the "raw" surface, and no improvement occurred, as the tincture of iodine could not penetrate the crust.

The remaining two of the seven patches were treated daily with equal parts of raw linseed oil and 3½ per cent. tincture of iodine and raw linseed oil respectively.

The raw linseed oil-tincture of iodine mixture caused a striking improvement within five days after the commencement of the treatment. The raw linseed oil caused the "lumps" to come off easily, thus exposing the "raw" surface to the action of the iodine.

The raw linseed oil alone had no effect other than softening the "lumps" and facilitating their removal.

EXPERIMENT II.

Transmission Experiments.

All infected material was obtained from Sheep 29146. The scrapings were made from the lesions on the skin after the "lumps" had been removed, and immediately transferred to the susceptible experimental sheep.

A.—Transmission by means of Scrapings.

(*a*) *Intact Skin.*

Sheep 27566.—Wool parted on right side of animal and lumpy wool-scrapings rubbed on the skin.

Result: In the course of the next four months no lesions developed.

(*b*) *Intact Skin + Moisture.*

Sheep 24694.—Treated as Sheep 27566 and patch moistened daily.

Result: 6th day.—Two pinhead-sized light yellow nodules appeared on the treated patch of skin; skin sensitive and reddened at base of the nodules.

7th day: Nodules bigger and contained a small amount of yellowish pus.

The nodules gradually increased in size and became covered with crusts, until on the twelfth day of the experiment these measured 0.5 cm. × 0.25 cm. × 1 cm. They were light brown in colour and extremely hard.

From the 13th day onwards improvement set in until the skin lesions had completely disappeared on the twenty-eighth day of the experiment.

(*c*) *Scarification of the Skin.*

Sheep 27566.—Wool parted on left side of animal and lumpy wool-scrapings applied to the slightly scarified skin.

Result: On the second day three pinhead-sized, light yellow nodules appeared on the treated patch. These nodules increased in size and contained a small amount of yellowish pus on the fourth day. Brown crusts began to form on the fifth day and increased in size until they measured 0.5 cm. × 0.25 cm. × 0.25 cm. on the twelfth day of the experiment.

Improvement set in on the fifteenth day and continued until complete recovery on the twenty-eighth day after the infected scrapings had been applied to the scarified skin.

(*d*) *Scarification of the Skin + Moisture.*

Sheep 24694.—Wool parted on left side and lumpy wool-scrapings applied to scarified skin and the treated patch moistened daily.

Result: On the second day, ten pinhead-sized brownish nodules had developed on the treated patch and the surrounding skin was swollen, reddened, and very tender. These nodules, which contained a small amount of pus on the third day, became covered with brownish crusts on the fifth day. They increased in size until two of them measured 1.0 × 0.5 × 0.75 cm. on the eleventh day of the experiment. The skin surrounding these lesions was sensitive and very hard.

Improvement set in from the sixteenth day and continued until complete recovery on the thirty-first day of the experiment.

B.—Transmission Experiments by means of Cultures prepared from the Skin Lesions caused by Lumpy Wool.

Smears and histological sections revealed the presence of a fungus in the skin lesions caused by lumpy wool. (See figs. 5 and 6.)

In transmission experiments (*b*), (*c*), and (*d*), it was possible to demonstrate a similar fungus in the skin lesions, which developed subsequent to the application of lumpy wool scrapings.

Dr. Scheuber, of the Department of Bacteriology, was successful in obtaining pure cultures of the fungus in serum bouillon.

Sheep 27566.—Pure cultures of the fungus in serum bouillon were poured on to three patches of the skin, two of which were lightly scarified. All three patches were moistened daily.

Result: The unscarified patch showed no changes in the course of the following five weeks.

On the second day, light yellowish nodules developed on the two scarified patches. On the fourth day, these nodules contained a small amount of pus. Brownish crusts formed from the fifth day and gradually increased until they measured 1 × .75 × 1 cm. on the twentieth day of the experiment.

Smears made from these lesions revealed the presence of a fungus in the tissues similar to the original one.

Improvement set in on the twenty-sixth day until complete recovery on the thirty-eighth day of the experiment.

C.—Transmission by means of Contact.

(*a*) The following susceptible young sheep (4-tooth) were placed in contact with Sheep 29146:—

Sheep 27542.
Sheep 28351.—Wool and back moistened once daily.

Sheep 29146 offered an excellent source of infection, as it was constantly shedding hard lumps of matted wool. Unfortunately, as already stated, it died on the sixth day after arrival.

In the course of the following eight months, Sheep 27542 developed no skin lesions.

On the 12th day of the experiment, Sheep 28351, which had been moistened daily, had developed a dry crustaceous eczema. The moistening was continued, with the result that the eczema grew worse, and the animal began to shed the wool on its back. There was rapid loss in condition. On discontinuation of the moistening, the eczema subsided, and there was complete recovery within 25 days after the moistening had been discontinued.

Smears prepared from this eczema did not reveal the presence of a fungus. This animal was kept under observation for 8 months and developed no skin lesions other than those described above.

Result: Negative.

(*b*) The following young sheep (4-tooth) were placed in contact with Sheep 24822:—

Sheep 22946.
Sheep 24820.

Neither of these two sheep developed any skin lesions during the course of the following 12 months.

Conclusions.

The skin lesions associated with the Australian and South African "lumpy wool" respectively appear to be identical and to occur under the same climatic conditions.

Heat and the degree of humidity apparently play a very important part as predisposing factors in the occurrence and spread of this disease. These conditions naturally favour the growth of fungi.

In the course of the above described experiments conducted under the conditions prevailing at Onderstepoort, at that time, the following was established:—

(1) A mixture of equal parts of raw linseed oil and tincture of iodine had a striking curative effect on lumpy wool.

It stands to reason that the best results will be achieved when the lesions are treated in the earliest stages of development. Care should be exercised in treating far advanced cases, because with daily treatment amounts of iodine might be absorbed which become dangerous. Such cases should preferably be slaughtered, as there is very little hope of recovery, and such animals only serve as sources of infection.

(2) Negative results were obtained by rubbing freshly collected lumpy wool scrapings on to the dry intact skin.

(3) The presence of a fungus was demonstrated in smears made from the lumpy wool lesions on Sheep 29146. (See figs. 5 and 6.)

(4) Lesions of a transitory nature, which resemble very closely those of lumpy wool, were produced by rubbing freshly collected lumpy wool scrapings on to (*a*) the moist intact skin, (*b*) the dry scarified skin, (*c*) the moistened scarified skin, and (*d*) by pouring pure cultures of the fungus on to the scarified skin.

In all these lesions, the presence of the fungus found in the lesions on Sheep 29146 was demonstrated.

(5) In-contact experiments gave negative results.

No definite conclusions can be drawn as to the negative results of the in-contact experiments, because the transmission of the disease from one animal to another depends to a considerable extent on climatic conditions.

(6) It was possible to produce an eczema with shedding of wool in a sheep by moistening the wool on twelve successive days.

Bull is of the opinion that the fungus lives as a saprophyte in colonies in the fleece of sheep, and when conditions are favourable it invades the skin. The beneficial effect of moisture in regard to the spreading of lumpy wool appears to be twofold, namely, rendering the conditions for fungus-growth very favourable and also softening the epidermis, and consequently facilitating its penetration by the fungus.

Recommendations.

In the light of the foregoing experiments and information, the following measures of combating the disease could be suggested:—

All affected animals should be isolated. Unfortunately, many cases, especially those in the earliest stages of development, are only detected at the time of shearing. Advanced cases should be slaughtered, whereas the earlier stages of the disease could be treated with a mixture consisting of equal parts of raw linseed oil and a $3\frac{1}{2}$ per cent. tincture of iodine.

It stands to reason that the most effective method of preventing the disease is to examine very carefully sheep introduced into a "clean" flock. It must, however, be kept in mind that sheep might harbour the causative fungus in their fleece without any "lumpy wool" lesions being discernible on the sheep.

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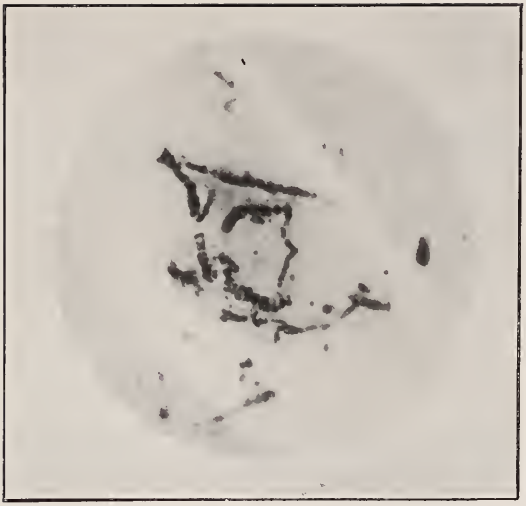

Fig. 5.—Smear made from lesion after removal of the " lump ". Stained with Giemsa. Magn. 1,000 ×

Fig. 6.—Smear made from lesion after removal of the " lump ". Stained with Giemsa. Magn. 1,000 ×

Actinobacillosis and other Complications in Sheep which may arise from the Feeding of Prickly Pear (*Opuntia* spp.)

By A. D. THOMAS, D.V.Sc., Veterinary Research Officer, Onderstepoort.

Prickly Pear as Fodder.

The prickly pear question has occupied the attention of our farmers and legislators in South Africa for very many years and can well be said to have become one of our national problems.

The problem is greatly complicated by the fact that two conflicting views exist regarding the utility of this plant; the one that it is a noxious weed, yearly encroaching on huge tracts of land to the exclusion of useful pasture and other vegetation; the other that it is a useful stand-by for stock in times of drought, especially the spineless varieties which are now widely cultivated for this purpose.

As a fodder the prickly pear has but a relatively low nutritive value; as a succulent, however, it makes extraordinary good use of the scanty rainfall and is capable of storing large quantities of water in its fleshy " leaves " and retaining it over long periods of drought.

This is the main reason why, apart from certain drawbacks such as thorns, etc., it constitutes such a god-send to tide stock over the all too frequent droughts in these regions.

It is for this reason, too, that the contemplated introduction of the prickly pear parasite (*Cactoblastis* spp.) which is so successfully destroying the prickly pear in Australia, has been prohibited, from fear that it may also exterminate the useful varieties of prickly pear in Africa. The value of prickly pear for cattle is, of course, fully appreciated by ranchers in other countries, e.g. Texas and Australia. The practicability of feeding it to sheep, however, had still to be demonstrated in South Africa. It is with this object in view that the Department of Agriculture in this country undertook to conduct experiments with various kinds of pear, including the wild thorny variety.

The last experiment of this type, to which most of these observations refer, was carried out at Graaff-Reinet, Cape Province. The situation chosen for this experiment was eminently suited for this trial, as the ground was literally overgrown with the common or thorny variety of prickly pear.

The objects of this experiment were mainly—

(*a*) to determine the feeding value of prickly pear for sheep from (i) the maintenance standpoint, (ii) the productive standpoint (wool and breeding);

(*b*) to determine the cost of clearing and reclaiming for pasture, land overgrown by the pear.

The results of this experiment have not yet appeared in print, but it is understood that they will be published in due course.

The material available and the opportunities for studying the effects of this plant were excellent. It seems unfortunate, therefore, that in this as in many feeding experiments, many aspects of the problem arising from the artificial conditions imposed on the animals, but having a direct and important bearing on the final results, were not sufficiently taken into account.

The following will serve as examples of what is meant in this respect:—

I. *Harmful Effects due to Physiological and Chemical Factors, quite apart from the Low Nutritive Value of the Plant.*—Amongst those which should receive attention one might mention methods of cutting up, as affecting ease in mastication, prevention of choking, and palatibility; acidity, fermentation, production of bloating, excessive and continuous purgation (scouring), intoxication and actual poisoning.

The scouring effect alone, for instance, deserves special mention since it was of a particularly persistent nature and affected all animals fed on the pear. It is difficult to believe that such continued purging can remain entirely harmless. In this connection a feature of the prickly pear which should be investigated, and which incidentally might prove of interest to the plant physiologist, is the presence of considerable amounts of calcium oxalate in the pear "leaves" in the form of so-called "Raphides." The occurrence of these structures in many plants is well known to botanists, but their significance is perhaps not yet satisfactorily understood. Chemical analyses of prickly pear are available, but these are apparently based on the ashed plant only, so that they do not reflect other possibly harmful organic substances such as oxalates, etc. Although calcium oxalate is such an insoluble salt and hardly likely to produce serious effects when ingested, there are as yet no experimental data regarding its physiological actions and ultimate fate during passage through the alimentary tract.

Messrs. Graf and Otto, of this Division, were good enough to carry out a few approximate oxalate determinations on prickly pear. They report having found from 0.5 to 0.68 per cent. of this substance expressed as oxalic acid in the fresh plant.

II. *Harmful Effects due to Traumatic Agents.*—The prickly pear is well protected by two types of thorns: the ordinary plain, long white thorns, and the minute bristles which by reason of their number and peculiar, barbed structure cause a lot of trouble by getting into the eyes, penetrating the lips, mouth, oesophagus and stomach wall.

There is little doubt that "sore mouth," a condition often encountered in sheep during the course of this experiment, can be so severe as to cause in itself considerable loss of condition.

III. *Harmful Effects due to Infectious Agents.*—These are usually secondary to the traumatic influences mentioned above, and the entry of such organisms is generally favoured by the lowered resistance of the animal. Under this heading the organisms of Lignières and Preisz-Nocard causing actinobacillosis and caseous lymphadenitis, respectively, seem to play a prominent part.

The Graaff-Reinet Experiment.

It is quite beyond the scope of this short article to discuss the objects and results of the feeding experiment itself. All that it is hoped to achieve, is to draw attention to some of the factors and complications mentioned above.

The incidence of actinobacillosis in this experiment seems of sufficient interest to warrant a brief description of its pathology and pathogenesis. It is the only one of the various complications mentioned that the writer was able to devote any attention to.

The feeding experiment was confined to sheep. Some 431 Merino ewes, 430 Persian ewes, and 87 Merino wethers were transferred from the Grootfontein School of Agriculture in March, 1927, and the feeding was actually started on 1st of April of that year.

Briefly, the sheep were divided into several lots and kept in bare paddocks.

Prickly pear "leaves" prepared in various ways were allowed *ad. lib.* to all animals, and were supplemented by a ration of about 12 oz. dry lucerne-hay per head per day.

It should be mentioned here that the sheep did not take to the prickly-pear habit very keenly at first, and had to be "taught" to eat the plant by withholding drinking water entirely.

Many different ways of preparing the prickly-pear were tried, e.g.:—

I. Thorns were singed off the standing plants with a blow-lamp, and the sheep "allowed" to graze on the patch so prepared. This method was very soon abandoned altogether because the sheep developed very bad "sore mouths" and, in the words of the attendant, "seemed to emaciate and pine away". It appears that these sheep much preferred the fruit that they could pick up than the singed leaf, and consequently fed largely on this, with the results noted. The fruit is not provided with thorns, but is covered with countless minute bristles, which were the cause of the trouble.

II. Singed "leaves" were also fed in troughs, but the sheep disliked this so much that they suffered badly from thirst, and this method had to be discontinued.

III. Maceration and fermentation was also tried in the hope that it would soften the thorns sufficiently to render them innocuous. This method had also to be abandoned, because the fermented leaf affected the sheep adversely, causing bloating, intoxication, and other alarming symptoms which are described as resembling those of geilsiekte.

IV. Another method of feeding and the one which was eventually adopted throughout the latter part of the experiment was simply to feed the freshly chopped up leaf.

The "joints" or "leaves" were gathered every day and sliced up by running them through a power-driven root-cutter. This more or less pulpy mass was then placed in troughs before the sheep, which were then able to consume up to 10–12 lb. of it per day.

In this way the thorny defence of the pear was broken, enabling the sheep to get at and nibble the fleshy portions of the leaf and so avoid injury to a certain extent.

This unfortunately does not apply to the small bristles. These minute barbed thorns are very numerous and so well protected that even singeing hardly affects them. Animals feeding on any part of the plant have no option but to chew and swallow masses of them.

Effects of Prickly-pear Feeding.

At the close of the experiment in 1930, the losses were as follows:—

Merino wethers: 9 out of 87 in $2\frac{1}{2}$ years.
Merino ewes: 85 out of 431 in $2\frac{1}{2}$ years.
Persian ewes: 49 out of 403 in $1\frac{1}{2}$ year.[1]
Total: 143.

Unfortunately, one is left in doubt from the report as to the actual cause of death in most cases. Findings such as jaagsiekte, geilsiekte, "opblaas", fever, poisoning, etc., when unsupported by laboratory examinations are inconclusive. On the other hand, the frequency with which abscess in the throat, lungs, stomach, and liver is recorded, is significant in the light of observations which follow.

The mortality in the lambs born in this experiment is even more striking, but scarcely unexpected, seeing that the ration provided was barely sufficient for maintenance of non-breeding animals. Most of the lambs were born weak and premature or died shortly afterwards from lack of nourishment and exposure. Quite a number are recorded as having choked through eating cactus and a few through abscess in the lungs.

The actual losses were as follows:—

Merino lambs: 333 out of 703.
Persian lambs, 287 out of 741.

(1) The Persians were transferred back to Grootfontein in October, 1928.

Actinobacillosis.

It was only towards the close of the experiment (November, 1929) that the writer paid a short visit to Graaff-Reinet and was able to collect the information and make the few observations which form the subject of this paper.

The local observations were of necessity rather curtailed by the short stay, but as regards the pathology of actinobacillosis, the writer was fortunate in being able to obtain preserved material and also several affected sheep which were transferred to this Institute for further examination.

Outward Appearances.

Clinically the sheep fed with the pear showed a general unthriftiness which in some cases amounted to extreme emaciation (see Fig. 1). At this stage sheep were fed with freshly pulped pear from troughs only. Many animals had thick, swollen, tough lips, due to scar formation and to numerous small abscesses, the result of thorn pricks. The tongue was often likewise affected. Small pustules and bleeding thorn pricks being visible or palpable both on the skin and in the mucous membrane or musculature. In many instances there were enlargement of, or nodules in the mandibular and retropharyngeal glands.

A peculiar feature was that on being exercised some sheep very soon developed laboured breathing accompanied by a harsh cough and râles. These latter symptoms explain why the condition was often mistaken for jaagsiekte. The dyspnoea in these cases was due to the enlarged, diseased retropharyngeal glands, causing pressure stenosis of the glottis and pharynx. The wool of the neck was usually matted together by the mucilaginous sap of the prickly pear and bristled with thorns.

Post-mortem Findings.

At autopsy of the few cases that were available the following lesions were found:—

Multiple small abscesses up to the size of a hazel nut in the skin, mucous membrane or musculature of the lips and tongue, generally disclosing the presence of a small spicule in the centre, when cut open. The lymphatic glands, particularly the retropharyngeal, then the mandibular, mediastinal and bronchial were often the seat of extensive chronic purulent processes. These were of two main types.

A. The Preisz-Nocard type with its well-known characteristic greenish, caseous mass and concentric formation in the gland, so well known that it is unnecessary to describe it here.

B. The actinophytic type, i.e. single or multiple centres of granulation tissue with burrowing, irregular cavities containing sticky, bright yellow pus. Nearly all the nodules seen or palpable in the lips, gums and tongue were of this type, usually with a small thorn in the purulent centre.

In the retropharyngeal, mandibular, bronchial and mediastinal glands the lesions were similar only much more extensive. The glands sometimes were greatly enlarged with burrowing pus cavities and connective tissue formation. No thorns were present so that infection was in all likelihood metastatic in nature. As has already been mentioned above, such enlarged glands caused great obstruction of the gullet and windpipe (see Fig. 2).

Local induration of the pharynx and oesophagus was also noted. It has also been reported that severe ulceration of the oesophagus and stomach have been seen by the attendants.

In the abomasum, especially at the pyloric ring, the mucous membrane was invariably studded with countless prickly pear spicules, often so close together as to simulate short bristles on a brush. Some of these were sometimes embedded in the mucous membrane in a small speck of pus. A form of chronic indurative bronchopneumonia appears to have been of very common occurrence in sheep that died in this experiment. The exact explanation of this lesion is not known, but very probably the condition is secondary to the stenosis of the larynx and difficulty in breathing.

In one case the lungs showed a patchy greyish colour with numerous purulent foci. This later proved to be a case of generalised actinobacillosis of the lung (see Fig. 3).

Histology.

Sections from the lesions of actinobacillosis presented very typical and uniform appearances. A considerable amount of granulation tissue usually formed a tough capsule for the nodules. Within this was an accumulation of neutrophiles and pus cells in which eosinophilic masses very similar to the ray fungus of actinomycosis were present. Very often these masses could be seen surrounding and distinctly associated with a small thorn. The ray-like effect is not at all as distinct as in the Streptothrix actinophyte, the edge of the "colony" being more irregularly broken and granular in appearance. There was no evidence of a central mycelium in any of the colonies. Furthermore, only the softer tissues were affected, never the bony structures.

Sections of the abomasum showed that the wall in many instances was riddled with spicules in all stages of penetration. Some were sticking on the surface of the mucosa, while others were embedded deeply in the mucous membrane or even in the muscular layers. Others again were surrounded by a zone of neutrophiles in which the actinophytic growth could always be found next to the thorn (see Fig. 4).

Bacteriology.

Bacteriological examinations were made in a few cases only. [I am greatly indebted to Dr. E. M. Robinson for his valuable co-operation in this respect.] A dozen sheep were transferred from the Experimental Station to this Institute and cultures made from the yellow pus in the enlarged retropharyngeal lymphatic glands of a

few. After several days at room temperature, a thick growth became apparent, which proved to be a pure culture of a short bacillus indistinguishable in all its characteristics from the Actinobacillus described by Lignières.

In subsequent cases, i.e. the remaining sheep killed six months and over, after having left the experiment, no such growth could be obtained from similar lesions. This in spite of the fact that actinophytic colonies could easily and distinctly be demonstrated in the nodules.

This fact would appear to support the view advanced recently by many authorities to the effect that the eosinophilic structures consisting of club-shaped rays, do not represent young sprouting forms of the fungus, but rather a protective shell secreted by the organism itself. This secretion becomes impregnated with certain salts and remains visible when stained in the usual way, even after the organism itself has died out.

It is interesting to note that even in the wall of the abomasum, actinophytic growth took place around the penetrating thorn. The causal organism must, therefore, be taken as capable of surviving the acid gastric juices.

Course of the Disease.

Of the dozen sheep transferred from the Experimental Station to this Institute a few were killed for bacteriological purposes, and the rest kept for various lengths of time in order to note the course of the infection. Lesions were to be found in all of these sheep in the form of small and large nodules or abscesses both in the lips and tongue and in the pharyngeal and mandibular lymphatic glands.

These sheep were in poor condition when they arrived, but soon after the change of environment and diet, they rapidly improved. The above-mentioned nodules instead of enlarging and extending, became smaller and more fibrous, the yellow pus became inspissated. After six months many of the smaller nodules had shrunk to mere cicatrices. In these older lesions the small thorn could often still be found and with it actinophytic masses. Although, as has been stated, cultures from them remained sterile.

It would appear, therefore, that the actinobacillus responsible for the lesions is only mildly pathogenic to the healthy, well fed animals. Under favourable conditions, i.e. semi-starvation and debility of the animal, the organism provided it can gain entry with a foreign body, sets up a chronic local abscess and may metastasise to the lymphatic glands or even become generalised in the lungs and elsewhere.

The histopathology of caseous lymphadenitis is too well known to need mention here. The pathogenesis of this organism is probably influenced by the same factors as actinobacillosis and this fact lends support to the findings of the Australian workers (Seddon *et al.*, 1929) who state that this organism enters the skin through abrasions, shear wounds, and with penetrating grass seeds, etc. The bacillus of Preisz-Nocard was isolated from the lesions in order to confirm the diagnosis.

The Prickly-Pear Thorns.

The prickly pear fed in this experiment was of the thorny variety which now grows wild in this region. Each axil on the "joint" usually carries three or more sharp, hard, long, white thorns so disposed that it is practically impossible to touch the fleshy portion with one's fingers without being stuck.

Apart from this already formidable defence the prickly pear has a further weapon in the form of tiny thorns known to botanists as "spicules" or "glochids." These minute thorns or bristles by reason of their structure, small size, and great numbers, give rise to a most disagreeable form of itch or sting which is well known to all who have ever attempted to pluck the fruit or handle the prickly pear in any way. These spicules are very numerous on the fruit but are also present on the "joints." Small bundles of them are encased in the many cup-shaped recesses or axils which stud the whole surface of fruit or leaf.

The structure of these spicules under the microscope is very impressive (see Figs. 5 and 6). They consist of slightly curved shafts of hard, amber coloured substance, with a very sharp point. The whole length of the spicule (1-3 mm.) is covered with delicate and well shaped barbs pointing backwards, so that once stuck the thorn can not readily be retracted and can, therefore, only penetrate deeper in the one direction.

These spicules are very loosely attached at their base so that the merest disturbance will dislodge them. In fact, shaking of the plant in dry weather causes them to shower down so that they often get into the eyes of stock, and cause severe inflammation. It is peculiar to note that in the so-called spineless variety of *Opuntia*, the spicules are much more numerous and fill the whole axil, apparently making up for the absence of the large thorn. It is interesting to note further that in young shoots of one season's growth, the spicules are entirely covered within the axillary recess, but they gradually protrude as the "joint" becomes older.

In numbers of course these spicules far exceed the thorns, they are less numerous in the wild thorny variety, but more so in the "spineless." (It has been computed that a single joint of the latter variety carries anything from 40,000–50,000 spicules.)

As the animal feeds, some of these spicules stick into the mucous membrane, so that the lips, tongue, pharynx, oesophagus and stomach become riddled with spicules penetrating to varying depths. Most of these minute thorns pass through the alimentary tract without doing much harm. Others penetrate the tissues and ultimately become embedded in connective tissue. Some, however, carry with them infection of some kind and not only does the thorn act as an inoculating agent, but actually gives protection to the pathogenic organism. The spicule, therefore, in spite of its size, is probably just as harmful if not more so than the thorn, on account of its numbers, structure and peculiar infection-carrying properties.

Discussion.

The tendency in many recent publications on prickly pear has been to boost this plant as a fodder and to overlook or underestimate

certain drawbacks that it possesses. While there can be no question as to the value of prickly pear, particularly the spineless variety, as a stock fodder, the Graaff-Reinet experiment has served to prove that too much must not be expected from this plant, nor must it be forgotten that in certain circumstances it may be dangerous.

In considering the mortality figures in this experiment, certain facts have to be borne in mind. The sheep were constantly confined in bare paddocks and had therefore no access to vegetation other than the daily ration of prickly pear and dry lucerne. Geilsiekte, or as it is now understood, hydrocyanic-acid poisoning, from other sources than the prickly pear, can thus be excluded.

There was no infectious disease present. In two cases only, was a diagnosis of jaagsiekte confirmed. Verminosis was conspicuous by its absence throughout.

It seems thus that, apart from normal wastage and accidents, all deaths can be attributed directly or indirectly to the bad effects of the prickly pear. It is true that these bad effects are probably exaggerated in an experiment of this kind because of the forced feeding of large quantities of pear with a minimum of concentrates and no water. For these very reasons, however, such an experiment should prove useful in bringing out dangers or errors which may be avoided or corrected if prickly pear has to be used as a fodder by force of circumstances.

The question of scouring, and intoxication by the prickly pear, can unfortunately not be taken up here since no actual experimental data are available, beyond the records of many deaths occurring under suggestive circumstances. The fact that such a high percentage of oxalate occurs in this plant should make the problem well worth investigating further.

Having noted the peculiar structure of the prickly-pear thorns and spicules, it is not at all surprising to see chronic purulent processes such as actinobacillosis and caseous lymphadenitis present to that extent. The spicules are ideal agents for inoculating micro-organisms and also for affording the local protection and irritation necessary to many chronic infections of that type. Whether the spicule actually carries infection with it from the plant or whether it merely serves as an inoculating agent to existing saprophytic organisms of the alimentary tract and skin, has not been determined. Magnusson in dealing with Actinomycosis is of opinion that the causal organism is present on the normal mucous membrane and may be inoculated by foreign bodies penetrating through the mucous covering. It is also possible that sheep have more delicate mouth parts than cattle and are, therefore, more liable to injury. Cattle certainly seem to be able to consume quite large quantities of prickly pear with impunity, when used to it, but it may yet be found that they also suffer from these chronic purulent infections.

Although spicules penetrate the tissues in such great numbers, it is surprising what little damage is done. In the first place not all spicules carry infection with them—they merely become embedded in connective tissue as foreign bodies. Secondly the virulence of the Actinobacillus is very low, it seems enhanced by low states of vitality

and malnutrition of animals. Most lesions produced by it tend to heal out spontaneously when not too far advanced and if the animal is subsequently well cared for.

Taking this experiment as the extreme of severity, namely in regard to low nutritive value of ration, absence of drinking water and maximum of prickly pear consumed, it must be admitted that the direct losses which are below 15 per cent. over a period of $2\frac{1}{2}$ years are not excessive. If one adds to this the loses in lambs and wool, which are indirectly due to the same causes, then the damage becomes considerably more serious.

As the bad effects of prickly pear will naturally depend largely on the amount consumed and length of time for which it is fed, it should be possible to minimise the risk of losses by adopting a reasonable course of action.

In the first place farmers should not be led to believe that a maximum of prickly pear with a minimum of other food is all that is required to keep their stock in health. Prickly pear should only be regarded as a good succulent supplement to a ration which must be otherwise balanced. Where possible drinking water should always be provided, and also a sufficient ration. In practice animals so treated will usually find enough scraps of grazing even in the drought, so that they will probably take in only as much prickly pear as is good for them. Given in this way, prickly pear will no doubt present the least possible risk of setting up dangerous after-effects. The fact that prickly pear is fed in any quantity only in winter and drought also further reduces these risks.

Other measures of a more general nature are of course obvious, namely to avoid overstocking, provide winter food reserves, plant spineless cactus and other drought-resisting succulents.

Reverting to the peculiar absence of verminosis in sheep in this experiment, it is well worth noting that these sheep, prior to their transfer from Grootfontein, were known to harbour gastro-intestinal parasites, such as wire- and nodular worms. They lost their infection one and all soon after being at Graaff-Reinet. It is, of course, well known that purgatives of any description will very often clear out nodular worm mechanically, even when not affecting the parasite itself. Prickly pear as is quite evident from the observation made, is a very effective purgative and moreover, the high oxalate content may have some effect on the parasites. Whatever the explanation for this fact may be, it is well worth recording, since we may still find in it a valuable, cheap, and effective vermifuge.

Summary.

1. Certain interesting features arising in a prickly-pear feeding experiment to sheep are described.

2. A rather high mortality among the experimental animals led to a cursory investigation being made, the results being briefly as follows:

3. Lesions of actinobacillosis were found to be very common. Deaths due to this cause were, however, not as numerous as those due to other factors.

4. Infection with this organism is shown to take place through the agency of the prickly pear spicule, a minute barbed thorn with which this plant abounds.

5. Actinobacillosis under these conditions was not found very virulent and sheep with early lesions when removed from prickly pear feeding and placed on good food generally recovered entirely.

6. Preisz-Nocard infection was also found fairly frequently. Infection probably also took place through the agency of the above mentioned spicules penetrating either through the alimentary tract or through the skin.

7. The majority of deaths in the adult sheep, however, were due to undetermined causes, the description and circumstances of death strongly suggesting intoxication or poisoning as the cause.

8. The large losses in lambs, over 40 per cent., can be attributed to the inadequate food supply for the mothers. Most of the lambs were born dead or died shortly afterwards from lack of milk. A small proportion died from more direct effects of the prickly pear.

9. The deaths due to undetermined causes mentioned under 7 are probably referrable to several factors.

A. *Predisposing Factors.*—Low nutritive value of pear and inadequate supplementary ration, no drinking water, breeding.

B. *Injurious Factors.*—Thorns and spicules causing more or less severe stomatitis, certain substances in the pear itself causing continuous scouring and probably capable of causing death by intoxication or simply by gradually wearing down the resistance of the animal.

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Fig. 1.—Sheep suffering from actinobacillosis of the retropharyngeal glands. Note emaciation and extended head.

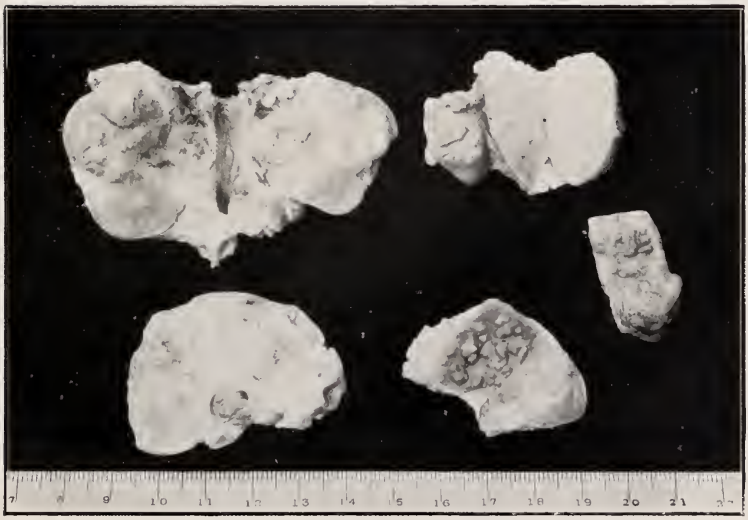

Fig. 2.—Retropharyngeal lymhphatic glands of three sheep affected with actinobacillosis.

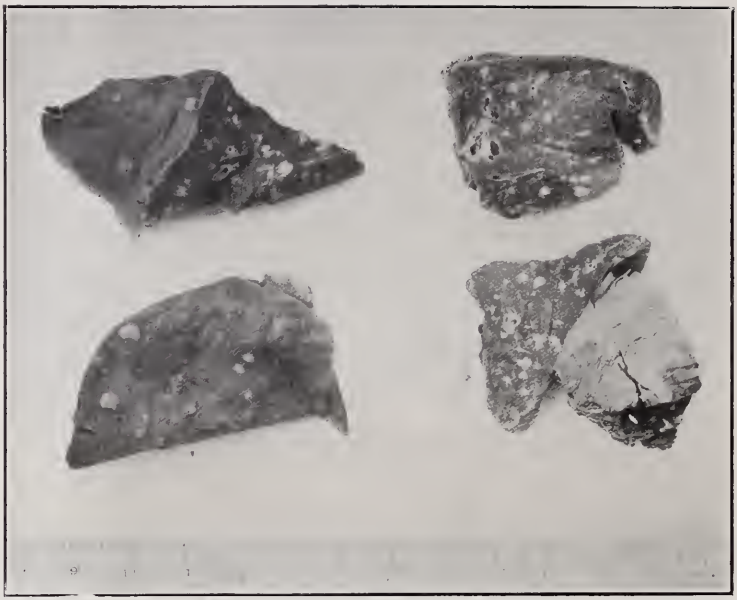

Fig. 3.—Portions of lungs of one sheep. Generalised actinobacillosis.

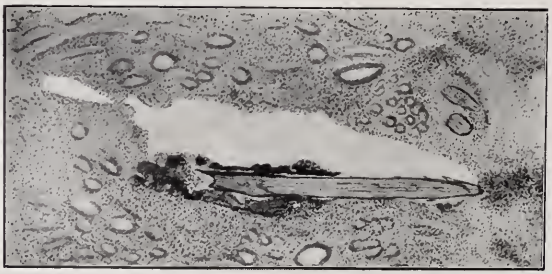

Fig .4.—Wall of abomasum with penetrating spicule surrounded by actinobacillus growth. 40 ×.

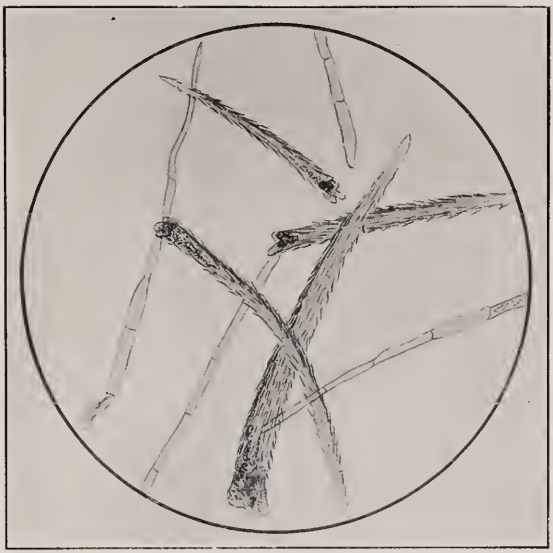

Fig. 5.—Spicules of prickly pear plant, to show barbed structure. 40 ×.

Fig 6.—As in Fig. 5, higher magnification. 200 ×.

Section IV.

Parasitology.

H. O. Mönnig ... Wild Antelopes as Carriers of Nematode Parasites of Domestic Ruminants. Pt. I.

H. O. Mönnig ... The Specific Diagnosis of Nematode Infestation in Sheep.

H. O. Mönnig ... The Development of Nematode Eggs and Larvae in Cattle Dung.

H. O. Mönnig ... A Second Species of the Nematode Genus *Acanthoxyurus*.

H. O. Mönnig ... Two New Species of the Nematode Genus *Hartertia*.

H. O. Mönnig ... Two New Nematodes from the Suricat (*Viverridae*).

G. A. H. Bedford New Genera and Species of *Mallophaga*.

Bernard Smith... A Study of the Sheep Blow-Flies of South Africa.

D. G. Steyn ... ... The Toxicity of the Pupae of the Moth *Nudaurelia cytheria*.

Wild Antelopes as Carriers of Nematode Parasites of Domestic Ruminants.—Part I.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

Little work has yet been done on the possibility of transmitting nematode parasites of antelopes to domestic ruminants and vice versa. In a country like South Africa, in which wild buck are frequently to be found on the same pastures as sheep and cattle, the poblem is undoubtedly a very important one. As the following article will show, there are probably still many unknown species of nematodes in antelopes, and it is surprising to what an extent they can be transmitted to sheep. The knowledge gained during the course of this work makes it imperative to continue the research in this field, and the writer hopes to be able to make a special effort in this direction.

Through the kind assistance of Dr. R. Bigalke, Director of the National Zoological Gardens at Pretoria, the writer was enabled to study most of the following cases. I take this opportunity to express my gratitude and appreciation to Dr. Bigalke for his assistance and keen interest in the work.

Arrangements were made whereby the writer was enabled to hold post-mortems on animals dying in the Pretoria Zoo. Most of these animals were the property of dealers and had been captured recently, so that the worm infection was still probably a natural one. The worms were carefully collected for identification, while the faeces from the colon of the host were taken and cultured. Larvae developing in these cultures were studied and given to lambs that had been suitably prepared, with controls where necessary. Owing to stress of other work, it was not possible to make as much out of every case as could have been done, but the information that was gained contains much that is new and important.

CASE No. 1.

Steenbuck (*Raphiceros rufescens*), female, 2-tooth.

Received 21.3.30 from Louis Trichardt, northern Transvaal.

Died 12.4.30 of traumatic peritonitis.

In the abomasum 9 males and 10 females of a worm morphologically identical with *Haemonchus contortus* were found. The faeces culture yielded no larvae as the carcass was rather decomposed.

CASE No. 2.

Blesbuck (*Damaliscus albifrons*), female, 2-tooth.

Received: 27.3.30 from Pretoria district.

Died: 17.4.30, killed on account of paralysis.

In the abomasum there were 19 males and 36 females of a worm morphologically identical with *Haemonchus contortus*, in the duodenum and jejunum 9 males and 10 females of a worm that proved to be a new species of the genus *Impalaia*, Mönnig, 1924 (*Heligmosominae*) described below. The faeces culture eventually also showed a small number of larvae apparently identical with the filariform larvae of *Strongyloides papillosus*. Besides these worms, there were found on the skin the ticks *Boophilus decoloratus* and *Rhipicephalus evertsi* and in the frontal sinuses 8 maggots identified by Mr. Bedford of this Institute as *Oestrus ovis*.

Impalaia nudicollis n. sp.

Slender worms of a light red colour, the females having a slight spiral twist.

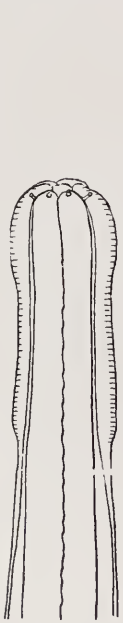

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1.—*Impalaia nudicollis*—anterior extremity.
Fig. 2.—*Impalaia nudicollis*—hind end of male.
Fig. 3.—*Impalaia nudicollis*—hind end of female.

The cuticle is slightly inflated around the anterior extremity (Fig. 1) and is transversely striated in this region, which is 0.14-0.15 mm. long in the females and 0.13-0.14 mm. in the males. The

rest of the cuticle bears 12 fine, longitudinal striations in both sexes. The tubercular appearance of the anterior extremity seen in *I. tuberculata* Mönnig, 1924, is not present in this species.

The mouth is surrounded by apparently three small lips (Fig. 1) which bear two lateral and four submedian papillae. The head is 0.046-0.059 mm. wide in the females and 0.051 mm. in the males. The oesophagus is cylindrical, 0.44-0.52 mm. long in the females and 0.44-0.48 mm. in the males.

Cervical papillae could not be found, nor could the excretory pore be located. The nerve ring is situated 0.33-0.39 mm. in the females and 0.37-0.41 mm. in the males from the anterior extremity.

The males are 7.5-8.2 mm. long and 0.11-0.12 mm. broad in front of the bursa. The bursa has large lateral and dorsal lobes, though the latter is not distinctly marked off. The rays are typical of the genus (Fig. 2), but the dorsal ray does not show the characteristic bend which is seen in the case of *I. tuberculata*. The ventral rays are divergent, almost equal and distally both bend forwards. The lateral rays are equal but the postero-lateral is longer than the others and the tips of the three rays are not close together. The externo-dorsal ray arises from the dorsal stem at about one third of its length from the base and is stouter than the same ray in *I. tuberculata*. The dorsal stem bifurcates near its end, each branch again bifurcates almost immediately and the two median branches each bear a small median protuberance. There are two equal filiform spicules 0.91-0.98 mm. long and a gubernaculum of simple shape 0.094-0.10 mm. long.

The females are 14.8-16.7 mm. long and 0.14-0.16 mm. broad. The hind end of the female narrows from behind the vulva and ends in a blunt, slightly knobbed point, 0.067-0.074 mm. behind the anus (Fig. 3). The vulva is situated near the anus, 0.24-0.26 mm. from the posterior extremity. The vagina runs forward and there is one ovejector, with a strongly developed pars haustrix, and one uterus and ovary. The eggs measure 0.063-0.074 × 0.039-0.046 mm. and are segmenting when laid.

Type host.—Blesbuck (*Damaliscus albifrons*).

Location.—Small intestine.

Locality.—Pretoria Zoo, recently from Pretoria District.

Type specimens in Onderstepoort helminthological collection No. 2265.

The faeces cultures of this buck yielded a fair number of mature larvae, which migrated upwards from the 5th day. The bulk of these larvae resembled those of *Haemonchus contortus*, while the rest were, as stated above, apparently filariform larvae of *Strongyloides papillosus* and a small number of unknown larvae, probably those of *I. nudicollis*. The latter larvae were not studied in this case since better material had been obtained from Case No. 3 described below.

Infection Tests.

Six year-old lambs, showing no *Haemonchus* larvae in recent faecal cultures, were given a double dose of Wireworm Remedy on the 5th and 6th May, 1930. On 10.5.30 lambs 24312, 24313 and 24326 were drenched with water containing the larvae from the culture of Case No. 2, while the other three lambs, 24314, 24317 and 24329 were kept in the same pen as controls.

Faeces cultures made of these lambs on 13.5.30 showed no *Haemonchus* larvae.

Cultures of faeces made on the 11th June showed *Haemonchus* larvae in all three infected lambs, one having a very heavy infection. Of the three controls one also showed a few *Haemonchus* larvae, while the other two had none. The result is, therefore, not quite clear, but it does seem as if some of the infection was acquired from the larvae of the blesbuck's wireworm. Two other lambs, used in connection with Case No. 3 below, had been treated for wireworm with the above six lambs and were kept in the same pen. In their faeces cultures made on the 14th June, there were no *Haemonchus* larvae, and they can also be considered as controls in this test.

No *Impalaia nudicollis* was found in these lambs.

CASE No. 3.

Blesbuck (*Damaliscus albifrons*), male, 6-tooth. Received from the Sommerville Estates, Theunissen, O.F.S., caught about 28.4.30.

Died: 5.5.30, cause unknown.

In the abomasum there were three females of a worm morphologically identical with *Haemonchus contortus* and 7 males and 23 females of an unknown worm, described below as *Bigalkea albifrontis;* in the small intestine a large number of *Impalaia nudicollis* were found. The frontal sinuses contained some 40 large maggots of an *Oestrus* species, but apparently not *O. ovis*.

Bigalkea albifrontis n. gen., n. sp.

Slender, straight worms with a reddish colour.

The cuticle is not inflated around the head and shows no transverse striation but only 24 longitudinal striations in both sexes. The head is 0.023 mm. wide in the females.

The mouth is surrounded by a small mouth collar and a few indistinct papillae. There is a small buccal cavity into which the anterior extremity of the oesophagus protrudes (Fig. 4). The latter is cylindrical, gradually widening backwards and has a characteristic twist just anterior to the nerve ring; its length is 0.77-0.85 mm. in the males, and 0.79-0.96 mm. in the females.

The cervical papillae are prominent, wedge-shaped like those of *Haemonchus contortus*, and are situated 0.36-0.40 mm. from the anterior extremity in the males, and 0.33-0.35 mm. in the females. The distances from the anterior extremity to the nerve ring and the excretory pore are respectively in the males 0.27-0.29 mm. and 0.30-0.33 mm. and in the females 0.27-0.31 mm. and 0.33-0.36 mm.

The *males* are 7.2-7.44 mm. long and 0.09-0.098 mm. broad. The hind end is provided with a bursa (Fig. 5) having large lateral lobes and a small dorsal lobe which has a deep central notch (Fig. 6) dividing it into two halves. The ventral rays are close together at their bases and at their tips but diverge in between; the latero-ventral is stouter than any other ray. The antero-lateral and the other lateral rays are widely divergent. The dorsal ray has practically no stem, giving off at its base the externo-dorsals and two small dorsal branches which diverge immediately, each being bidigitate. The dorsal lobe of the bursa with its rays is rather small and difficult to see.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 4.—*Bigalkea albifrontis*—anterior end.
Fig. 5.—*Bigalkea albifrontis*—male bursa, lateral view.
Fig. 6.—*Bigalkea albifrontis*—male bursa, dorsal rays.

Prebursal papillae are well developed. There is no accessory bursal membrane and the genital cone is simple.

There are two equal, slender spicules, 0.172-0.196 mm. long, each ending in three tips of which the middle one is the largest (Fig. 7). A gubernaculum is present and is 0.066-0.074 mm. long.

The *females* are 9.9-11.3 mm. long and 0.10-0.11 mm. broad. The tail is 0.13 mm. long, the hind end narrows down gradually and ends in a fine point.

The vulva is situated 1.69-1.83 mm. from the hind end and has prominent lips. On these lips and on other places in the vulvar region, usually dorsally and laterally on the left side (Fig. 9), there are prominent cuticular thickenings which bear distinct transverse striations. The vagina and the ovejectors are strongly developed; the uteri are opposed. The eggs are segmenting when laid and measure 0.070-0.078 × 0.035-0.046 mm.

Host.—Blesbuck (*Damaliscus albifrons*).

Location.—Abomasum.

Locality.—Pretoria Zoo, recently from Theunissen, O.F.S.

Type specimens in Onderstepoort Helminthological collection No. 2267.

Fig. 7.

Fig. 8.

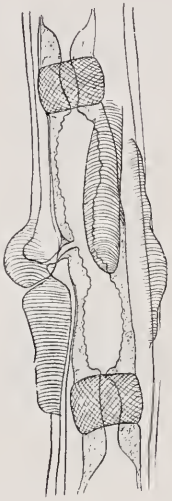

Fig. 9.

Fig. 7.—*Bigalkea albifrontis*—spicule and gubernaculum.
Fig. 8.—*Bigalkea albifrontis*—female, hind end.
Fig. 9.—*Bigalkea albifrontis*—female, vulvar region.

These worms are fairly closely related to the genus *Ostertagia* but differ from the species of that genus, especially in two important points, viz., the absence of an accessory bursal membrane and the nature of the dorsal ray in the male bursa.

Diagnosis: *Bigalkea*-Trichostrongylinae: slender worms with a small head and about 24 longitudinal striations on the cuticle; cervical papillae present. Male: Bursa with large lateral lobes and a dorsal lobe which is deeply notched in the middle; ventral rays close together but not parallel; antero-lateral diverges from the other laterals; externo-dorsal arises from base of dorsal stem, the latter bifurcates at the base forming two small divergent rays which are

bidigitate at their extremities. Accessory bursal membrane not present. Spicules short, equal, ending in three processes; gubernaculum and prebursal papillae present. Vulva in posterior quarter; oviparous; parasites of mammals.

Type species: *Bigalkea albifrontis* n. sp.

The faeces cultures of this buck yielded about 3 million larvae, which migrated upwards in the culture jar under suitable conditions from the 5th day. The larvae were all alike and resembled those few seen in Case No. 2 which were considered to be the larvae of *Impalaia nudicollis*. Considering the worms which were recovered from the two cases there can be little doubt that the larvae belong to this species. Larvae which could be those of *Bigalkea albifrontis* were not seen, but since the adults were few in number their larvae may have been missed, although in the various tests made a very large number of larvae were seen under the microscope. Unfortunately, larvae not migrating upwards were not searched for in the culture medium.

Infective Larvae of *Impalaia nudicollis*.

Morphology.—The larvae are enclosed in a sheath which probably is the skin of the second ecdysis and measures 0.717-0.757 mm. in length. The greatest breadth is 0.027 mm. The mouth opening is a small pore surrounded by apparently four papillae, standing dorsal, ventral and lateral and there are on each side a pair of amphids (Figs. 10 and 11). The mouth opening leads into a narrow pharyngeal tube which is 0.016 mm. long. The oesophagus is cylindrical, 0.16-0.18 mm. long, and is dilated interiorly forming a swelling 0.008 mm. long, conspicuously pigmented with light-brown granules, and possessing anteriorly a cup-shaped depression into which the pharyngeal tube is fixed. The nerve ring and the excretory pore are situated 0.102 mm. and 0.114-0.125 mm. respectively from the anterior extremity. The intestinal cells are filled with granules as usual. The genital primordium could not be found and must be very small and inconspicuous. The sheath has a long, fine tail, measuring 0.145-0.160 mm. from the anal opening, while the tail of the larvae inside the sheath is only 0.045 mm. long and ends in two distinct points appearing as if a wedge-shaped piece had been cut out of the tail (Fig. 12). In many of these larvae a small quantity of opaque matter lies between the two points of the tail.

These larvae are, therefore, easily identifiable on account of the long tail of the sheath, the double point of the larval tail and the pigmented anterior swelling of the oesophagus.

Bionomics.—The larvae are negatively *geotropic*, as they migrate upwards in the culture jar under suitable conditions. They are positively *heliotropic* to a mild diffused light as can be seen from the conditions required for migration. *Direct sunlight* causes the movements of the larvae to stop after a short time; after one hour of exposure about 2 per cent. were dead in a watch-glass of water—these tests were carried out during the winter and the temperature of the water did not rise much.

Placed in a watch-glass of water on a block of ice, the larvae sank to the bottom after a while and became quiescent; small bits of ice were added to bring the temperature to 0° C.; after 3 hours exposure the larvae all started swimming again on being warmed.

Fig. 10.

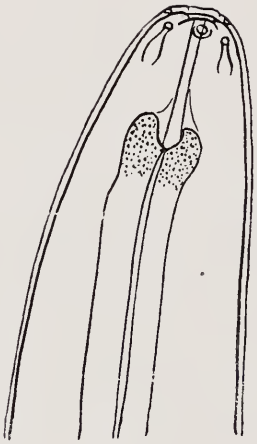

Fig. 11.

Fig. 12.

Fig. 10.—*Impalaia nudicollis*—anterior end of infective larva.
Fig. 11.—*Impalaia nudicollis*—head of infective larva.
Fig. 12.—*Impalaia nudicollis*—hind end of infective larva.

The larvae in a watch-glass of water are neither definitely attracted nor repelled by the point of a hot needle held against the bottom of the glass, although the heat makes them more active; they are, therefore, not positively *thermotropic*. To test resistance to desiccation, larvae free of debris were placed in drops of water on

slides, about 10,000 larvae per slide, and allowed to dry at room temperature. A specimen was then moistened at intervals. Although these tests were done during the cold season, when the air could, therefore, not absorb much water and the actual dryness of the larvae could not have been severe (see Mönnig, 1930), the number of larvae recovering decreased rapidly. After 2 hours practically all recovered. After 24 hours about 50 per cent. recovered; after 2 days about 10 per cent.; after 6 days about 1 per cent.; and after 22 days 0 per cent.

Infection Tests.

Two year-old lambs, Nos. 24320 and 24327, which had been given a double dose of Wireworm Remedy on the 5th and 6th May, and showed only a few *Trichostrongylus* and *Strongyloides papillosus* larvae in their faeces cultures, were drenched on the 13th May with water containing about 20,000 *Impalaia nudicollis* larvae in each case. The lambs were kept in a clean gravelled pen; there is little danger of accidental worm infection under these conditions during the winter months and none whatever where such parasites of buck are concerned, although that would not matter even if it should take place. To assist the establishment of the worms in the sheep neither salt nor any concentrates were given, which is a routine procedure when sheep are being infected experimentally with any worm.

Faeces cultures made of these two lambs on the 14th June showed no change from the previous infection. Cultures made on the 21st June, and also later showed no change and it thus appears as if *Impalaia nudicollis* is not transferable to sheep.

CASE No. 4.

Waterbuck (*Cobus ellipsiprymnus ellipsiprymnus*), female, 2-tooth. Captured in the northern Transvaal about 10.5.30.

Died: 24.5.30 of strangulation.

In the abomasum there was 1 male *Haemonchus bedfordi*; in the small intestine there were 2 males and 1 female of a hookworm described below as *Bunostomum dentatum* n. sp., numerous specimens of *Cooperia fuelleborni* Hung, 1926, a small number of worms which are here described as *C. fuelleborni* var. *hungi* n. var., a small number of *Trichostrongylus instabilis* with variations described below; in the large intestine 8 males and 4 females morphologically identical with *Oesophagostomum columbianum*, although slightly larger in all measurements, and a large number of oesophagostome nodules in the small and large intestines.

Haemonchus bedfordi le Roux, 1929.

Le Roux's types of *H. bedfordi* are not satisfactorily preserved and hence his description is not quite accurate, especially with reference to the spicules of the male. In this and in the following waterbuck very good material was obtained and a description of the spicules is given here. The structure of the spicules can be

accurately seen only after they have been removed from the body of the worm and with the application of slight pressure, in order to separate the two spicules from each other. Each spicule (Fig. 13) has two slender processes originating in front of the middle of the spicule and running parallel to the main portion, nearly reaching the distal extremity. These processes support the large terminal alae which are typical of the spicules of this species.

Fig. 13.—*Haemonchus bedfordi*—male spicules.

On examining spicules of *H. contortus* in a similar way, it was found that the left spicule also has a similar, though more slender process. These processes appear to be thickenings in the spicular alae and, since the alae of *H. bedfordi* are larger than those of *H. contortus*, one could expect this stronger development of supporting structures. The rest of le Roux's description of the spicules and his measurements agree with the findings of the present writer.

Bunostomum dentatum n. sp.

This description is based on 2 males and 1 female from the above waterbuck and 12 females from another waterbuck (see Case No. 5 below). The worms are morphologically typical specimens of the genus but differ from the other species of *Bunostomum* in several points.

The worms were blood-red in colour and were attached to the mucous membrane of the small intestine.

The cuticle is transversely striated at intervals of 0.002 mm. and is fairly thick.

There is a strong buccal capsule of the usual type opening anterodorsally and the mouth opening is surrounded by the usual two lateral and four submedian papillae, the lateral having each a slender prolongation on the dorsal plates (Figs. 14 and 15). The length of the buccal capsule along its dorsal wall is 0.137 mm. in the males and 0.16-0.18 mm. in the females. Ventrally there are two large cutting plates at the oral margin, joining laterally with the thinner dorsal plates. The dorsal tooth is strong and clearly shows the duct of the dorsal oesophageal gland passing through it. A typical feature of this species is that the two ventral lancets are very strong and somewhat hood-shaped (Fig. 15). At the base of these lancets the posterior, ventral border of the capsule is thick and frequently has a deep external groove as shown in Fig. 15. There is a well-developed oesophageal funnel with a strong chitinous lining.

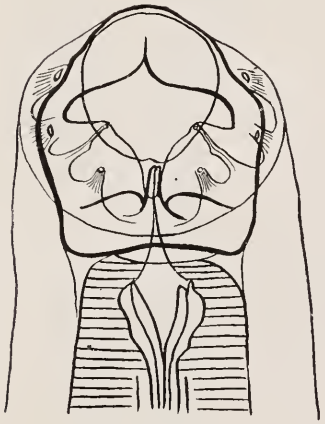

Fig. 14.

Fig. 15.

Fig. 14.—*Bunostomum dentatum*—head, dorsal view.
Fig. 15.—*Bunostomum dentatum*—head, lateral view.

The *males* are respectively 12.4 and 12.7 mm. long with a maximum thickness of 0.27 mm. The oesophagus is 0.87-0.95 mm. long and is, as usual, club-shaped. The distance from the anterior extremity to the nerve ring is 0.56 mm. and to the excretory pore and the cervical papillae 0.67 mm. The bursa (Fig. 16) is well developed and asymmetrical. The rays are arranged very much as in the type species. The right externo-dorsal arises very close to the base of the dorsal stem, while the left arises close to the point of origin of the two dorsal branches. The latter end each in three unequal digitations. There are two equal, slender spicules, 0.637 mm. long, slightly twisted and bearing small alae. The gubernaculum is 0.135 mm. long and of simple shape, wider anteriorly than posteriorly.

The *females* are 19-20.4 mm. long, with a maximum thickness of 0.48 mm. The oesophagus is 1.2-1.3 mm. long. Distance from anterior extremity to nerve ring is 0.6 mm. and to cervical papillae and excretory pore 0.65-0.75 mm. The anus is situated 0.48-0.58 mm. from the posterior extremity and the tail ends with a somewhat blunt point (Fig. 17). The vulva is situated 5.7-6.9 mm. from the anterior extremity; the ovejectors and uteri present no special features. The eggs found in the females were not further developed than the 2-cell stage and measured 0.055-0.7 × 0.039 mm.

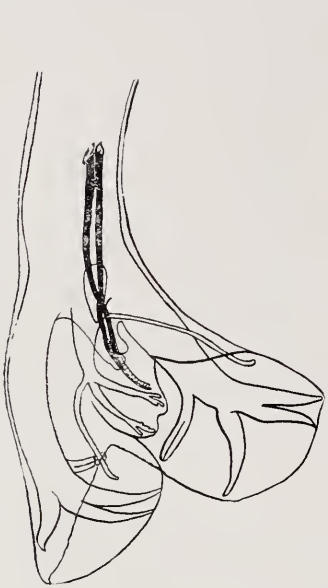

Fig. 16.

Fig. 17.

Fig. 16.—*Bunostomum dentatum*—male bursa and spicules.
Fig. 17.—*Bunostomum dentatum*—female, hind end.

Host.—Waterbuck (*Cobus ellipsiprymnus ellipsiprymnus*).

Location.—Small intestine.

Locality.—Pretoria Zoo, recently from northern Transvaal.

Specimens in Onderstepoort Helminthological Collection No. 2272.

Cooperia fuelleborni Hung, 1926.

This worm was described by Hung (1926) from a waterbuck that died in the Hamburg Zoological Garden. The present writer found it again in the same host. Schwartz (1928), in his review of the Genus *Cooperia* states that he considers the differences between this worm and *C. curticei* not to be sufficient to warrant the erection of a

new species. There are, however, distinct differences, and the present writer must uphold the species *C. fuelleborni*. Through the kindness of Drs. M. C. Hall and A. Hassall of the United States Bureau of Animal Industry, the writer obtained specimens of *C. curticei* for comparison, which settled the uncertainty. A drawing is given of the spicules of *C. fuelleborni* (Fig. 18) since the alae have not been completely illustrated by Hung. The spicules of many Trichostrongyles are difficult to represent in drawing, since their appearance varies much with the direction from which they are seen and many have alae that have not been represented in published drawings. Hung gives the length of the spicules as 165-185μ; the writer found many specimens with even longer spicules, up to 0.196 mm. A drawing (Fig. 19) shows the male bursa of this species. The genital cone is complicated as in other species of this genus.

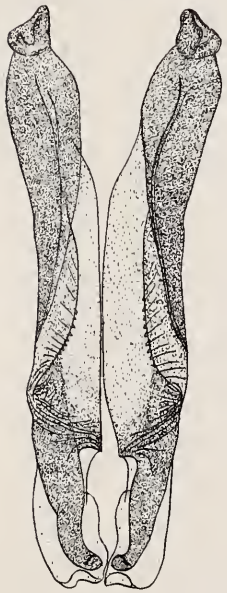

Fig. 18.

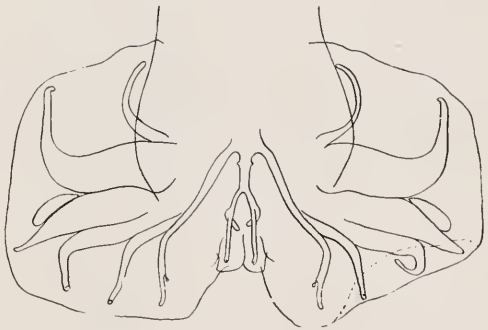

Fig. 19.

Fig. 18.—*Cooperia fuelleborni*—male spicules.
Fig. 19.—*Cooperia fuelleborni*—male bursa.

Cooperia fuelleborni var. *hungi*, n. var.

While studying the Cooperia specimens from this buck, a number of males with longer and somewhat different spicules were readily picked out from the material of *C. fuelleborni*. These males are in general slightly larger but otherwise very similar to *C. fuelleborni*, also in the structure of the bursal rays. No constant differences were found in the specimens of females, which would allow the females of this new variety to be picked out. On account of this great general

similarity, it was decided to describe this worm as a variety of *C. fuelleborni* and not as a new species. The spicules (Fig. 20) measure about 0.235 mm. in length; the distal extremities are not bent ventrad as much as in *C. fuelleborni* and the transverse ridges coming from the serrated fold do not continue on to the distal thickened points of the median alae. A comparison of Figs. 18 and 20 will give a much better idea of the spicules than can be given in a description. The bursa shows the same two transparent structures resting on the antero-lateral rays as drawn in the case of *C. fuelleborni* (Fig. 19).

Fig. 20.—*Cooperia fuelleborni*, var. *hungi*—male spicules.

Variations in *Trichostrongylus instabilis*.

The material of this species found in the waterbuck and also later in sheep infected from this buck shows a series of variations in the spicules from the typical spicules of *T. instabilis* to a type which, when found alone, might lead one to think that it is a definite variety or even a new species. The series of variations is, however, continuous and the lengths of the spicules vary from 0.125 mm. to 0.158 mm. Ransom (1911) gives the length of the spicules of *T. instabilis* as 0.135-0.145 mm. The chief differences are that the terminal hook of the spicules varies in length from the usual—about one quarter of

the length of the spicule—to only about one half of that length, while the concavity of the spicule immediately anterior to this hook is also sometimes much greater than usual.

Kalantarjan (1928) described from the sheep *T. skrjabini* as a new species. The writer can unfortunately not read the Russian text, but from the drawings it seems possible that this is also only a variation of *T. instabilis* with short terminal hooks to the spicules.

The faeces cultures of this buck yielded a fair number of larvae which migrated upwards on the fifth day under suitable conditions. The majority of these larvae appeared to be those of a *Cooperia*, while a very small proportion were larvae of *Oesophagostomum*, and some *Trichostrongylus* larvae were also seen. Infection tests with these larvae will be described together with those of the next case.

CASE No. 5.

Waterbuck (*Cobus ellipsiprymnus ellipsiprymnus*), female, 4-tooth.

Captured in northern Transvaal about 10.5.30.

Died 24.5.30 of toxaemia from infected wounds.

In the abomasum were found numerous *Haemonchus bedfordi* and a few *H. contortus*. The small intestine contained 12 females of *Bunostomum dentatum* and one full-grown *Moniezia expansa*. This examination was made in a failing light, and some worms may have been missed.

The faeces cultures yielded a fair number of larvae of *H. contortus*, a larger number of unknown larvae " X " (later found to be those of *H. bedfordi*), and some larvae of *Trichostrongylus* but no *Bunostomum* larvae. Larvae not migrating upwards were unfortunately not searched for in the culture medium.

Infection Tests (Cases 4 and 5).

Six cross-bred (Merino and Persian) lambs, about 4 months old, were given a double dose of Wireworm Remedy on the 2nd and 3rd June. On the 10th June Nos. 25596 and 25607 were given larvae from Case No. 4. Nos. 25603 and 25611 were given larvae from Case No. 5, Nos. 25609 and 25610 were kept as controls in the same pen.

Faeces cultures made of these lambs on the day of infection showed only rare larvae of *Trichostrongylus* and *Strongyloides papillosus*. Faeces cultures made 3 weeks after infection showed, in addition, a few *Cooperia* larvae in 25596. Cultures made 4 weeks after infection showed, in addition to the original infection, *Cooperia* larvae in 25596 and 25607 and *Haemonchus* and " X " larvae in all four infected lambs, the latter more numerous in 25603 and 25611 than in the other two, while the controls showed no change.

Lamb 25607 was killed on the 21st July. It had about 30 adult *H. bedfordi* and a few *H. contortus*, numerous *C. fuelleborni* and its variety *hungi* and 10 specimens of not quite mature *O. columbianum*.

Lamb 25603 was also killed on the 21st July. It had about 40 adult *H. bedfordi*, a few *H. contortus*, very few *C. fuelleborni* and some *T. instabilis*.

Lamb 25611 was also killed 21st July. It had a fair number of adult *H. bedfordi*, some *H. contortus* and *C. fuelleborni* with its variety *hungi* and also some not quite adult *O. columbianum*.

From this it can be concluded, that *Haemonchus bedfordi*, *Cooperia fuelleborni* and its variety *hungi*, have been transmitted to sheep. All three are new as parasites of sheep. From the cultures of these lambs the infection has also been carried further to other sheep with success.

Oesophagostomum columbianum was found in lamb 25596, infected from Case No. 4, which contained this worm, and the age of the specimens is just right, but a similar infection was also found in lamb 25611 infected from Case No. 5 which did not harbour this parasite and the result is consequently not clear; the infection may have been accidental.

It now remained to discover the identity of the larva " X ". The post-mortem findings of the three lambs that were killed, correlated with their faeces cultures, would indicate that these were the larvae of *Haemonchus bedfordi*. The larvae, however, differ so much from those of *H. contortus*, having much longer tails to the sheath, that this assumption required more proof. (The larvae are described in the next article in this Report.) Four lambs were infected from the cultures of 25611 and 25603 and eventually showed the larvae " X " together with larvae of *H. contortus*, *Cooperia* and *Trichostrongylus* in their cultures and at post-mortem yielded the same species of worms as the lambs from which they were infected. About 200 of the larvae " X " were then picked out by means of a fine pipette under a binocular microscope and given to a clean lamb 29103 on the 29th August. The lamb was killed 4 weeks later and had in the abomasum about 60 adult specimens of *H. bedfordi*, and in the small intestine a few specimens of *Trichostrongylus instabilis* which were probably acquired accidentally. There can thus be little doubt that the larvae " X " are those of *Haemonchus bedfordi*.

CASE No. 6.

Springbuck (*Antidorcas marsupialis*), female, 2-tooth No. 28764. Bought 26.5.30 from a dealer. Caught in northern Transvaal about 3 weeks previously and transferred to Onderstepoort after a short stay at the Pretoria Zoo in a box.

Faeces cultures of this buck showed at first only larvae of *Trichostrongylus falculatus* (see next paper in this Report) and *Nematodirus*. The faeces also contained ripe segments of a *Moniezia*.

The animal was given larvae of *Haemonchus contortus*, *Trichostrongylus instabilis* and *Strongyloides papillosus* from sheep and later showed the larvae of these worms in its faeces cultures, while *Cooperia* larvae also began to appear. The *Cooperia* infection may have been very recent, but it is not clear why the larvae were not seen at an

earlier date if the infection had been acquired before the animal was captured. It had been standing in a box in the Zoo, not close to any buck other than springbuck, and it may have become infected from these.

The animal died of a broncho-pneumonia on the 6th August, and at post-mortem showed the following worms: In the abomasum a fair number of *H. contortus* and *T. instabilis*; in the small intestine *Nematodirus spathiger*, *T. instabilis*, *T. falculatus*, *Strongyloides papillosus*, two *Moniezia expansa* and a fair number of two new species of *Cooperia* described below as *C. serrata* and *C. antidorca*. The peritoneal cavity contained 27 specimens of *Cysticercus tennicollis*.

Three species of worms have, therefore, been transmitted to this buck from sheep, while the buck itself harboured four species known as parasites of sheep, and two new species. The latter were subsequently transmitted to sheep as will be described.

The Springbuck is one of the commonest antelopes kept on farms in many parts of the country, but it would seem that there is some danger in this practice, and it would be advisable to keep any kind of buck, which is being protected, in special camps away from sheep.

Cooperia serrata n. sp.

The Cooperia material collected from this springbuck contained two species mixed. The males could easily be separated, but the females were very much alike except that some had vulvar flaps and the others had none. The males and females were picked out separately and counted, and it was found that there were 25 males of the species named *C. serrata* and 82 females with vulvar flaps and 12 males of the species named *C. antidorca* and 43 females without vulvar flaps. Since *C. serrata* is closely related to *C. nodulosa*, Schwartz, 1928, the female of which has a vulvar flap, it was decided that the males and females probably belong together as their numbers seem to indicate.

Cooperia serrata is a typical member of the genus. The anterior two-thirds of the body in both sexes is coiled in a loose spiral. The cuticle bears ten longitudinal lines of the usual structure and is slightly inflated in the cephalic region where it has a delicate transverse striation. The cervical papillae are very small, 0.26 mm. from the anterior extremity in the male and 0.39 mm. in the female. The head is 0.041 mm. wide in the male and 0.044 mm. in the female, the width varying slightly with the cuticular expansion. The oesophagus is simple, 0.386 to 0.5 mm. long in the male and 0.46 to 0.5 mm. in the female. The nerve ring is situated 0.193 mm. from the anterior end in the male, and 0.27 mm. in the female, and the excretory pore 0.22 mm., and 0.32 mm. in the male and the female respectively.

The *males* are 6.9 to 7.8 mm. long and 0.106 mm. broad in the posterior quarter of the body. Prebursal papillae are present but are very small. The bursa is well developed and the rays are fairly

typical of the genus (Fig. 21). The medial surfaces of the lateral lobes bear fine dentations. The dorsal ray branches distally, each branch again dividing into a short, median, bidigitate branch and a lateral, rather slender branch which is directed postero-laterally. In this respect this species shows much similarity to *C. nodulosa* Schwartz, 1928. The spicules are also very similar in these two species, but they have only 4-5 dentate processes in *C. serrata* (Fig. 22), while in *C. nodulosa* there are 7 such processes. The spicules of *C. serrata* are 0.259-0.270 mm. long, brown in colour and more darkly pigmented in the posterior than in the anterior half. Each spicule has a strong dorso-medial arm, separated from the body, which bears 4-5 dentate processes, and a ventro-medial spine. The distal extremity of the body of the spicule is foot-shaped and concave, the " toe " directed medially and the " heel " laterally. The spicule bears two median and several large distal alar expansions which are not pigmented, and which can be seen properly only in spicules that have been dissected out. Fig. 22 was drawn from such a specimen.

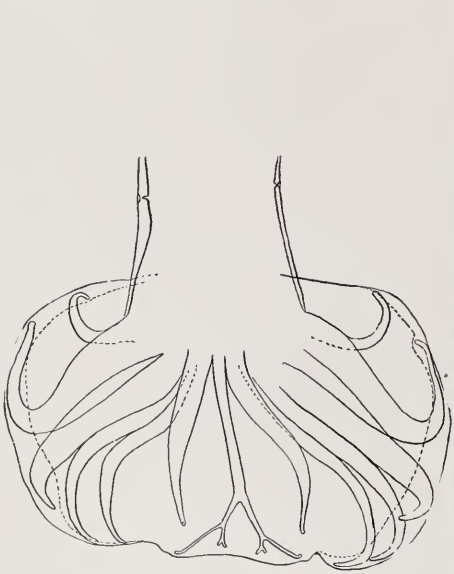

Fig. 21.

Fig. 22.

Fig. 23.

Fig. 21.—*Cooperia serrata*—male bursa.
Fig. 22.—*Cooperia serrata*—left male spicule, dorsal view.
Fig. 23.—*Cooperia serrata*—hind end of female.

The *females* are 8.8 to 10.3 mm. long and 0.113 mm. broad just behind the vulva. The tail is about 0.15 mm. long, tapering to the end which has a thin, finger-like point (Fig. 23). The vulva is covered by a linguiform flap and is situated 1.77 to 2.05 mm. from the tail end. The genital organs present no special features. Unfortunately no females contained eggs in a sufficiently advanced stage of development to allow measurements being made.

Host.—Springbuck (*Antidorcas marsupialis*).

Location.—Small intestine.

Locality.—Onderstepoort; recently from Pretoria Zoo and northern Transvaal.

Types in Onderstepoort Helminthological Collection No. 2285.

Cooperia antidorca n. sp.

The anterior portion of the body is curled in a loose spiral and the cuticle has ten longitudinal ridges. The cephalic cuticle is slightly inflated and transversely striated posteriorly, the first annulus being rather marked, separating the cephalic inflation into two portions. The diameter of the head is about 0.047 mm. in both sexes. Cervical papillae are small, 0.32 mm. from the head end in the male, and 0.33-0.38 mm. in the female. The nerve ring and the excretory pore are respectively 0.23 mm. and 0.29 mm. from the head end in the male and 0.25 mm. and 0.27-0.34 mm. in the female. The oesophagus is 0.35-0.4 mm. long in the male and 0.46 mm. in the female.

Fig. 24.

Fig. 25.

Fig. 24.—*Cooperia antidorca*—male bursa.
Fig. 25.—*Cooperia antidorca*—male spicules.

The *males* are 6.2 to 7 mm. long, and 0.113 mm. thick. The bursa has rather coarse dentations on the medial surfaces of the lateral lobes. The rays present no special features except the dorsal ray, which bifurcates about half way to the bursal margin, each branch being bidigitate (Fig. 24). Prebursal papillae are present but small. The spicules (Fig. 25) have a number of folds, ridges and alae, but no serrations nor dentations. They are 0.192-0.196 mm. long. A gubernaculum is not present.

The *females* are 7.7-9.7 mm. long and 0.106-0.121 mm. thick. The tail is about 0.159 mm. long and acute. The vulva is surrounded by slight thickenings of the cuticle which bear transverse striations, but there is no flap. It is situated 1.6-2.03 mm. from the posterior extremity.

Host.—Springbuck (*Antidorcas marsupialis*).

Location.—Small intestine.

Locality.—Onderstepoort, recently from Pretoria Zoo and northern Transvaal.

Types in Onderstepoort Helminthological Collection No. 2286.

Infection Tests.

Lambs 29102 and 29104 were given larvae from the cultures of Springbuck 28764 on the 11th August, 1930, and were then kept under conditions designed to prevent accidental infection. Cultures were made daily of the faeces of these lambs from the 25th August onwards. At first they showed only a few *Trichostrongylus instabilis* and *Strongyloides* larvae. *Cooperia* larvae began to appear in the case of 29102 in the cultures made 17 days after infection and in the case of 29104, 19 days after infection. *Trichostrongylus falculatus* larvae began to appear 21 days after infection.

On the 1st September lamb 29104 was killed. The abomasum contained numerous *Haemonchus contortus*. The small intestine contained a large number of *T. falculatus*, some *T. instabilis* and *Strongyloides papillosus*, a fair number of *Nematodirus spathiger* and *Cooperia serrata* and a smaller number of *C. antidorca*. All these worms, except *T. instabilis* and *Strongyloides papillosus* have thus been transmitted from the Springbuck.

Another springbuck, No. 28765, which had been bought together with the previous one, was also artificially infected with *Haemonchus contortus* and *Trichostrongylus instabilis*. A lamb infected from this buck showed *Cooperia serrata* and *Trichostrongylus falculatus*, which were also recovered from the buck itself when it died later. It had been attempted to infect this buck with *Oesophagostomum columbianum*, but at the post-mortem of the buck only nodules were found in the intestines and no worms.

The following list gives the new hosts and new parasites according to the above experiments, and also includes some specimens in the Onderstepoort Helminthological Collection not yet reported upon, since they were received after the compilation of the check list published by the writer in 1928. Those transmitted to sheep are marked with one asterisk, those transmitted from sheep with two asterisks:

Blesbuck (*Damaliscus albifrons*).

*Haemonchus contortus**
Impalaia nudicollis
Bigalkea albifrontis
Strongyloides papillosus

Bushbuck (*Tragelaphus sylvaticus*).

Haemonchus contortus.

Nyala (*Nyala angasi*).

Setaria labiato-papillosa.

Reedbuck (*Redunca arundinum*).

Oesophagostomum columbianum.

Rhebuck (*Pelea capreolus*).

Oesophagostomum columbianum.

Sheep (*Ovis aries*).

*Haemonchus bedfordi.**
*Cooperia fuelleborni.**
*Cooperia fuelleborni hungi.**
*Cooperia serrata.**
*Cooperia antidorca.**
Setaria hornbyi.

A single adult female specimen of *S. hornbyi* was recovered by Dr. A. D. Thomas of this Institute from the peritoneal cavity of a sheep at a post-mortem made here. There can be no doubt about the origin of this specimen. This is apparently the first record of a *Setaria* from sheep.

Springbuck (*Antidorcas marsupialis*).

*Haemonchus contortus***
*Trichostrongylus instabilis***
*Trichostrongylus falculatus**
*Strongyloides papillosus***
*Nematodirus spathiger**
*Cooperia serrata**
*Cooperia antidorca**
Moniezia expansa.

Steenbuck (*Raphiceros campestris*).

Haemonchus contortus.

Waterbuck (*Cobus ellipsiprymnus ellipsiprymnus.*)

*Haemonchus contortus**
*Haemonchus bedfordi**
*Cooperia fuelleborni**
*Cooperia fuelleborni hungi.**
Trichostrongylus instabilis.
Oesophagostomum columbianum.
Bunostomum dentatum.
Moniezia expansa.

After the experiments recorded above had been completed, an article appeared by le Roux (1930), who shortly afterwards left this Institute, in which he lists a number of wild antelopes and worms which he considers to have transferred from them to lambs. These worms include in most cases *Haemonchus contortus*, *Trichostrongylus instabilis* and *Oesophagostomum columbianum*, which are common in sheep at this Institute. Le Roux states that he made faecal cultures from antelopes in the Pretoria and Johannesburg Zoological Gardens " and the larvae thus obtained were fed to young lambs known to be free of helminths."

Since the present writer was not aware of le Roux's work on this subject and has not had the opportunity of seeing his experimental records, it is not possible to draw any conclusions from the short published note.

Summary.

One new genus, four new species and one new variety of Trichostrongylid nematodes are described: *Bigalkca albifrontis* and *Impalaia nudicollis* from *Damaliscus albifrons, Cooperia serrata* and *C. antidorca* from *Antidorcas marsupialis* and *Ovis aries, C. fuelleborni hungi* from *Cobus ellipsiprymnus* and *Oris aries.* Experiments on the transmission of worms from antelopes to sheep and vice versa are described, followed by a list of antelopes and worms constituting new hosts and new parasites. Besides those mentioned above, the following are new parasites for *Oris aries*: *Haemonchus bedfordi, Cooperia fuelleborni, Setaria hornbyi.*

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17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

The Specific Diagnosis of Nematode Infestation in Sheep.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

Several of the infective larval stages of sheep nematodes have been described by various authors, but these descriptions are scattered over a number of journals which may not always be available to all helminthologists; several species have not been described and there is no comparative account of these larvae, which would be very useful when one wishes to identify a number of different species of larvae occurring in a mixture.

After having acquired a fair amount of experience with larvae of sheep nematodes, the writer thought it desirable to publish the following brief account of available larvae, especially in order to put the diagnosis of worm infection in sheep on a sounder basis, with reference to anthelminthic tests.

It has repeatedly been mentioned by Hall and undoubtedly felt by many others, that the methods employed in testing the efficacy of drugs against parasitic worms have in the past been very unsatisfactory and there is even now much room for improvement.

Hall has developed a method of critical testing, by which our definite knowledge of the action of a number of drugs has been much improved. After the administration of the remedy the worms passed are counted; the animal is then killed and the worms left after treatment are also counted. The efficacy of the remedy is expressed as a percentage based on the two counts.

This method is a great improvement on other methods, though, as Hall has also pointed out, there are sources of error; e.g., some worms are digested and not passed out. The present writer also feels, that it is somewhat misleading to express results in percentages when less than 100 individuals are dealt with. To say that a remedy is 33 per cent. effective when it expels one of three worms is not justified, for one has no knowledge of what might have happened if there had been 100 worms present.

The methods employed in testing drugs against worms in sheep are still very unsatisfactory. Some workers make egg counts before and after treatment, then kill the sheep and, if the number of eggs have decreased, the drug is said to have acted on that species of worm of which there are few at the autopsy. Since the eggs of several of the sheep nematodes cannot be differentiated from one

another, this method of testing is completely unreliable and very misleading. Others treat a number of sheep and then kill them together with some untreated controls. The controls are supposed to indicate the original infection and the treated sheep what worms have remained after treatment and on this basis the conclusions are made. Now it is a fact, that in any flock of sheep the individual infections vary, sometimes very markedly, and a method of this nature can give more or less reliable results only when a fair number of sheep is used with each drug.

In order to obtain definite results by testing drugs on a small number of sheep, it is necessary to know exactly what infection the sheep have before treatment. If this can be determined, it can also be done after treatment, and it will not be necessary to kill the sheep, which is often a considerable advantage and means that the test is a much more economical matter. The same lot of sheep can be used repeatedly; they can be infected with larvae of the species in question, it can be determined whether the infection has established itself, the sheep are then treated, re-examined and again infected for another test.

For this purpose it is essential that the infective larvae of the different species should be well known and this paper is intended to assist other workers in this direction, in the hope that our methods of drug testing against sheep nematodes may be placed on a sounder basis.

Before the larvae are discussed, the writer would like briefly to sketch the procedure adopted in this laboratory in testing drugs against worms in sheep.

If sheep infected with the species of worm in question are not available, which is frequently the case, a number of suitable young sheep are infected from the cultures of a sheep with a stock infection. During this time it is advantageous to give little concentrated food and no salt to the sheep. Some species of worms can be given in large numbers; *Haemonchus* and *Trichostrongylus* are usually given at the rate of 1,000 larvae every second day until 4-10,000 or more have been given. Others, like *Oesophagostomum,* must be given at the rate of 50 every second or third day until 1-2,000 have been given, else diarrhoea results and the infection is not successful.

When the worms can be mature and have started laying, faeces are collected from the sheep and cultured in large fruit jars. For a detailed description of this procedure see Mönnig (1930). The cultures are kept at a suitable temperature (26° C.), preferably in a dark place, for 7 to 10 days. They are then placed in a warm room on a wooden table in a mild diffused light and the migrating larvae soon appear on the walls of the jars. When several hours have been allowed for migration, the cultures are classified roughly as strong, medium or weak, according to the number of larvae on the walls. A sample of the larvae from each culture is then examined by placing it in a drop of water on a slide, heating the drop gently until the larvae straighten out, which is a sign that they are dead, another drop of water is added and the slide examined under a low power of the microscope. The larvae can be counted to determine the percentages of the different species, but usually an estimation

is sufficient, since very exact percentages are not necessary. Each culture is then recorded against the number of the sheep in a special "culture book" which is useful for future reference, giving the percentages of the different larvae and the strength of the culture.

Some larvae (hookworms, *Nematodirus*, *Dictyocaulus*) do not migrate very well and if they have to be taken into account it is necessary to place a sample of the culture material into a Baermann separating apparatus in order to find these larvae. The *Nematodirus* and *Gaigeria* eggs could of course be recognized in making egg-counts, and *Dictyocaulus* infection is best determined by floatation methods applied to fresh faeces as for eggs.

Experience shows that the strength of a culture, unless it is strong, is not always a true reflection of the degree of infection. The conditions in the faeces may sometimes stop the development of a large proportion of eggs and a culture that should have been strong shows up as weak. This is a weak point of this method, but, keeping this in mind, it need not affect the usefulness of the method in preliminary tests, since it is found that all species are usually equally affected and not that one species may be totally suppressed while the others develop. In final tests or tests that have to be very critical, it is always advisable to make an egg-count simultaneously with the culture and to do this over three successive days each time. Such an egg-count would then reveal the true strength of the infection and would indicate whether the strength of the culture is correct. For methods of egg-counting in sheep faeces see Stoll (1930) and Mönnig (1928). It also sometimes happens that no larvae develop in a culture, without apparent reason. The examination must then of course be repeated, while an egg-count would show the true position. It is, therefore, absolutely necessary to check cultures by means of egg counts for definite results, but the egg count alone is not sufficient, as only the culture can show which species are present and in what proportions.

Usually drugs are tested against one species at a time, although it is useful to record the effects of the treatment on all species present. Only such sheep are therefore selected that show a sufficiently strong infection with the desired species, while the others are again given larvae.

The treatment is then carried out and, where it is possible, faeces are collected and worms passed are recorded, although in sheep even worms like *Oesophagostomum columbianum* are often killed and not seen in the faeces, because they probably decompose quickly in the large intestine, especially when the sheep do not purge.

Since many drugs cause a temporary decrease or total suppression of egg production, it is necessary to wait 12-14 days after treatment before the faeces are again examined. Now it is important to draw no definite conclusions from weak cultures of sheep which showed strong cultures before treatment. Usually, however, one tests a number of drugs and the faecal cultures after treatment clearly show that there is little change. If one or other drug seems to have acted, a re-test with egg-counts can then be made. In this way the writer has tested numerous drugs against *Trichostrongylus* spp. and *Oesophagostomum columbianum*, and only rarely was it found

necessary to kill a sheep to obtain special information, while useless drugs showed up clearly and useful drugs were soon detected so that they could be carefully re-tested. (These results will be published at a later date.)

This method, of course, entails a fair amount of work, especially when egg-counts are included, but no good results will ever be obtained without labour, and it is a waste of time and material to make tests which can give no useful information. With a little practice it is quite an easy matter to learn to recognize the different species of larvae, and rarely sheep harbour so many different species, whose larvae are rather similar, that the diagnosis becomes difficult for that reason.

As stated previously, most of the larvae discussed here have been described before by other authors, while others have not. In every case, however, the drawings and measurements have been made by the present writer, usually from a large amount of material.

There are many characteristics whereby the larvae can be recognized, but in the examination of cultures one desires to have certain points of comparison which will lead to quick recognition of different species. It is, therefore, intended to give here only salient points which are characteristic and not to describe minute anatomical differences. A table of certain measurements is appended. In each case the measurements show the most frequent (not the average) followed by the shortest and the longest obtained. This is useful to obtain certainty in cases of doubt, but the experienced worker will not often find it necessary to take measurements. It would be advisable to work out the equivalents of these measures in terms of the divisions of the ocular micrometer and the lenses used constantly, so that no further calculations need be made thereafter. The few measurements given are those that have been found to be the most useful in differentiating the larvae. Others, like the positions of the nerve ring, the excretory pore and the thickness of the larvae are generally not of much use in this connection. The length of the larval tail and the tail of the sheath, as well as the structure of the larval tail, are of the greatest importance.

1. *Strongyloides papillosus* (Fig. 1).—The filariform larva can always be easily recognized by the fact that it is very slender, it has no sheath and the oesophagus is very long, showing as a transparent streak reaching almost to the middle of the body. When the cultures are older and the reserve food granules in the intestine have decreased, the opacity of this part of the body is not so marked any more and the typical appearance of the larva is no longer so obvious. The tail has a bifid extremity.

Although this paper is restricted to the infective larval stages, it must be mentioned that one occasionally finds larvae, as well as males and females of the free-living generation of *Strongyloides* on the walls of the culture jar. These can easily be recognized by their short, stumpy shape with a sharply pointed tail (except in the male) and a rhabditiform oesophagus.

2. *Trichostrongylus instabilis*, *T. rugatus* and *T. axei* (Fig. 2). —These larvae are sheathed and of medium size, i.e. about 0.7 mm. long. The tail of the sheath is very short, about 0.097 mm., and

the tail of the larva itself ends in a slight knob-like thickening with one or two small points. This latter characteristic is very typical and can always serve to give certainty in a case of doubt. It is generally found that the infective larvae of the different species of a genus are very similar and usually impossible to differentiate from one another. In the case of *Trichostrongylus* the larvae of the species which are known to the writer are very much alike, except those of *T. falculatus*. From the drawing given by Sandground (1929) of a larva from human faeces, the adult of which was unknown, one can say with a fair degree of certainty that this must be a species of *Trichostrongylus*.

3. *Trichostrongylus falculatus* (Fig. 3).—On the whole very much like the larvae of other species *Trichostrongylus*, but the tail of the sheath is slightly longer (0.114 mm.) and may lead to suspicion that this is an *Ostertagia larva*, until the tail of the larva itself is examined.

4. *Ostertagia* (Fig. 4).—Larvae of *O. circumcincta* and *O. trifurcata* were available for study. The larvae are sheathed and fairly large, but their size does not make them conspicuous. The larval tail ends in a rather blunt point, which frequently has a minute hyaline tip. In many cases the sheath narrows rather suddenly behind the end of the larval tail and thus presents under a low magnification the same typical appearance as the tail of a *Trichostrongylus* larva. The tail measurements are, however, slightly larger than those of this species and lie in between the latter and those of a *Cooperia* larva.

5. *Cooperia* (Fig. 5).—Representatives of this genus are not frequent in sheep in South Africa, and the following description applies to the larvae of *C. fuelleborni*, *C. serrata*, and *C. antidorca*, which have been transmitted by the writer from wild antelopes to sheep and which must, therefore, be considered as parasites of sheep (see previous article in this Report). The infective larvae of these three species are very much alike, and it is probable that the larvae of other species of the genus will be similar. The larvae are sheathed and of medium size. The tail of the sheath, measuring usually 0.129 mm., is straight in larvae killed over a flame and is not very thin; the tail of the larva itself also tapers evenly to a point, which is not very acute. The *Ostertagia* and *Cooperia* larvae are the most difficult ones to differentiate, especially when they occur together with *Trichostrongylus* or *Haemonchus*.

6. *Haemonchus contortus* (Fig. 6).—Sheathed larvae, of medium size. The tail of the sheath is usually 0.149 mm. long and has a definite thin posterior portion and frequently shows a slight kink just behind the end of the larval tail. The latter runs to a fairly fine point.

7. *Haemonchus bedfordi* (Fig. 7).—This worm has also been transmitted to sheep by the writer (see previous article in this Report). The infective larva, however, has a longer tail to the sheath (0.160 mm.) than is the case in *H. contortus*, the extremity of the tail being very slender, rather resembling that of the sheep hookworm larvae.

8. *Bunostomum trigonocephalum* (Fig. 8).—The infective larvae of this species as well as those of *Gaigeria pachyscelis* are conspicuously shorter than the other larvae already described; usually they are more transparent, as there are not so many food granules in the intestinal cells and the posterior extremity of the oesophagus shows a more conspicuous bulbar swelling than in the other larvae. They can, therefore, be easily recognized under a low magnification The infective larvae of *B. trigonocephalum* is usually 0.598 mm. long. The tail of the larva is simple and the tail of the sheath, usually 0.145 mm. long, is very fine in its posterior quarter.

9. *Gaigeria pachyscelis* (Fig. 9). In general resembles the larva of *Bunostomum trigonocephalum*, but the tail of the sheath is longer, usually 0.172 mm., and also very fine in its posterior quarter. It may be very difficult to differentiate between these latter two larvae, but since both are hookworms and probably for most practical purposes very similar in their habits and reactions to drugs, this difficulty is of no great practical consequence. Moreover, the *Gaigeria* eggs are very large (115-118μ × 50-55μ). The presence of this species could be determined by examining faeces for eggs by a floatation method.

10. *Oesophagostomum columbianum* (Fig. 10).—This larva has a very typical appearance and cannot easily be mistaken for any other except the larva of *Chabertia ovina*. It appears to be of medium size, although the body looks somewhat shorter than that of the other sheathed larvae described above, while on the other hand, if the larva is measured, including the tail, it is found to be rather longer than the others, usually 0.83 mm. Characteristic are the coarse and very conspicuous transverse stration of the sheath, especially when the larva does not lie straight, as is often the case, and the long, whip-like tail of the sheath, which runs out into a slender, filamentous part. The tail of the larva itself ends in a simple point. The larva of a *Nematodirus* is much larger and the larval tail does not end in a point. It must be stated here that one occasionally finds *Oesophagostomum* larvae of which the slender portion of the tail has broken off and this may cause trouble unless one is aware of the possibility; the coarse striations of the sheath will cause suspicion and under a higher magnification it will be seen that the tail is broken off, ending as if squarely cut off.

11. *Chabertia ovina* (Fig. 11).—Owing to the scarcity of this parasite in South Africa the writer has had little opportunity of studying these larvae. On the whole they are very similar to those of *Oesophagostomum columbianum*. The infective larva has been well described by Cameron (1926). From the description and drawing of this author and the measurements of the present writer (obtained from a small number of larvae only) it seems as if the tail of the sheath is not as long as that of *O. columbianum*. The larvae studied had a slight dull yellow colour and the striations of the sheath were not very marked, while the *O. columbianum* larva is very transparent and the sheath usually shows conspicuous wrinkles as has been noted above. Cameron states that the intestiue has 32

cells. In the few specimens seen by the writer the intestine had 24 cells, while in the *O. columbianum* larva the intestine has 20-24 cells.

12. *Nematodirus spathiger* (Fig. 12).—This is a large larva, 1.158 mm. long, which is, therefore, easily recognized in a mixture of different species. The tail of the sheath is very long, usually 0.340 mm., and whip-like. The larval tail ends in dorsal and ventral wedge-shaped points with a slender finger-like prolongation between them, but this prolongation is seen only under a fairly high magnification.

13. *Dictyocaulus filaria*.—This larva does not belong to the gastro-intestinal group, but is included here since it may be found in cultures. As stated previously, it is best found by examining fresh faeces by a floatation method, as for eggs, and it does not migrate well in cultures since it is rather inactive. The larva has a characteristic knob-like thickening on the anterior extremity of the sheath, it usually has a brown colour (in the intestinal cells) and the tail is fairly short.

The following key may assist in the identification of these larvae. It may not hold for some species not considered in this paper, but belonging to the genera mentioned. The larvae of *Dictyocaulus filaria* and of *Chabertia ovina* are not included:—

1. Without sheath, oesophagus more than one-third as long as the body ... ... *Strongyloides papillosus.*
   Sheathed, oesophagus not so long 2

2. Total length less than 0.65 mm. 3
   Total length more than 0.65 mm. 4

3. Oesophagus with distinct bulbar swelling; tail of sheath with slender posterior extremity, less than 0.157 mm. long, usually 0.145 mm. ... *Bunostomum trigonocephalum.*
   Similar, but tail of sheath over 0.157 mm. long, usually 0.172 mm. ... ... *Gaigeria pachyscelis.*

4. Tail of larva not ending in a simple point 5
   Tail of larva ending in a simple point 6

5. Tail of sheath long, larva large ... *Nematodirus spathiger.*
   Tail of sheath short, larva of medium size ... ... *Trichostrongylus* spp.

6. Tail of sheath over 0.2 mm. long, sheath frequently coarsely striated ... ...*Oesophagostomum columbianum.*
   Tail of sheath less than 0.2 mm. long 7

7. Tail of sheath over 0.145 mm. long 8
   Tail of sheath less than 0.145 mm. long 9

8. Tail of shearth 0.16 mm. long or longer ... ... *Haemonchus bedfordi.*
   Tail of sheath less than 0.16 mm. long, usually 0.15 mm. ... ... *Haemonchus contortus.*

9. Tail of sheath more than 0.125 mm. long, *Cooperia* spp.
   Tail of sheath less than 0.125 mm. long, *Ostertagia* spp.

While this article was being prepared for the press, a publication by Morgan (1930) appeared on the same subject. This author described differentiating characters of nine species of larvae and also gives a description and measurements of the eggs of a number of species which can be differentiated on that basis. Morgan states that he can find no difference between the larvae of *Chabertia ovina* and *Oesophagostomum venulosum*. Larvae of the latter species have not been studied by the present writer. According to Morgan the tail of *Bunostomum trigonocephalum* is longer than that of *Haemonchus contortus*. Perhaps this observation may be due to shrinkage during the killing of the *Haemonchus* larvae, because the observations of the present writer on a very large number of specimens do not bear this out. Morgan states that " Since the position of the anus is often difficult to determine it was found more convenient to measure the distance from the tip of the tail of the third-stage larva to the tip of the tail of the second stage. This gave quite a reliable measurement, provided no undue contraction had taken place in killing the larva ". It is unfortunate that the author has resorted to this compromise, because the anus can be easily seen when it is situated at the edge of the body and, if not, the larva can be rolled over by a touch applied to the cover-slip. Moreover, two measurements which are both characteristic, i.e. the lengths of the larval tail and the tail of the sheath, are more valuable than the difference between them, which may not be characteristic. Nöller and Schmid (1927) and Nöller (1927) evolved a method of classifying the eggs of nematodes in fresh sheep's faeces according to the number of embryonic cells they contain. This method may be quite useful to a certain extent for preliminary work, but would be of little use in authelminthic tests, since too many species fall in the same group.

It is highly desirable that comparative studies of the infective larvae of parasitic nematodes of other animals should be carried out. Such studies have been attempted in the case of the horse by Heuber (1926) and by Poluszynski (1930), although both authors dealt with only a few species. On account of the large number of closely allied worms that have to be dealt with in the case of the horse, the study will be a difficult one, but it may perhaps be possible to distinguish the larvae of different genera and to throw some light on the grouping of species in the large genus *Trichonema*.

TABLE OF MEASUREMENTS OF LARVAE.

(All measurements in microns.)

	Strongyloides papillosus.	*Trichostrongylus instabilis, axei, rugatus.*	*Trichostrongylus falculatus.*	*Ostertagia circumcincta, trifurcata.*
Total length	637 (598-675)	694 (656-772)	714 (598-753)	907 (888-907)
Oesophagus	239 (227-259)	172 (151-181)	157 (149-161)	176 (172-188)
Genital cell	337 (314-373)	360 (345-400)	353 (333-368)	360 (353-411)
Tail, larva	94 (86-97)	67 (63-71)	63 (59-71)	78 (71-86)
Tail, sheath	—	97 (86-110)	114 (102-117)	117 (110-121)

	Cooperia, spp.	*Haemonchus contortus.*	*Haemonchus bedfordi.*	*Bunostomum trigonocephalum.*
Total length	810 (772-830)	733 (694-772)	753 (714-772)	598 (560-637)
Oesophagus	165 (161-165)	141 (127-145)	141 (141-145)	165 (153-165)
Genital cell	353 (306-368)	360 (353-380)	340 (333-360)	294 (274-294)
Tail, larva	71 —	67 (63-71)	67 (67-71)	63 —
Tail, sheath	129 (129-137)	149 (145-165)	160 (160-176)	145 (133-153)

	Gaigeria pachyscelis.	*Oesophagostomum columbianum.*	*Chabertia ovina* (Average).	*Nematodirus spathiger.*
Total length	618 (598-637)	830 (791-849)	772 —	1158(1119-1177)
Oesophagus	165 (149-168)	165 (157-172)	172 —	235 (227-235)
Genital cell	278 (255-282)	345 (337-360)	353 —	509 —
Tail, larva	63 —	74 —	67 —	59 (43-59)
Tail, sheath	172 (161-181)	223 (204-235)	172 —	340 (325-340)

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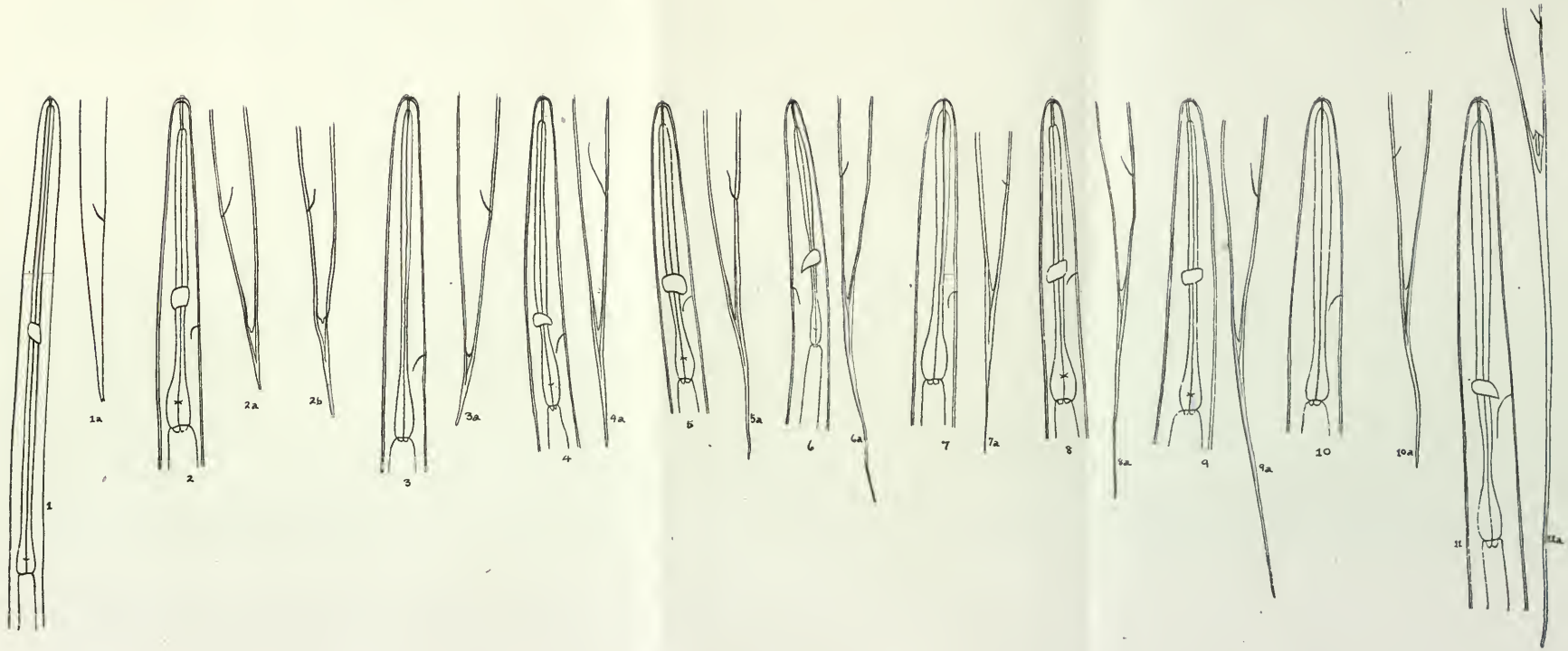

EXPLANATION OF FIGURES.

Figs. 1, 1*a*.—*Strongyloides papillosus.*
Figs. 2, 2*a*.—*Trichostrongylus* spp.
Fig. 2*b*. —*Trichostrongylus falculatus.*
Figs. 3, 3*a*.—*Ostertagia* spp.
Figs. 4, 4*a*.—*Cooperia* spp.
Figs. 5, 5*a*.—*Haemonchus contortus.*
Figs. 6, 6*a*.—*H. bedfordi.*
Figs. 7, 7*a*.—*Bunostomum trigonocephalum.*
Figs. 8, 8*a*.—*Gaigeria pachyscelis.*
Figs. 9, 9*a*.—*Oesophagostomum columbranum.*
Figs. 10, 10*a*.—*Chabertia ovina.*
Figs. 11, 11*a*.—*Nematodirus spathiger.*

The Development of Nematode Eggs and Larvae in Cattle Dung.—Preliminary Note.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

During the early Spring of 1930 a severe infestation with gastro-intestinal nematodes was noticed to occur in the cattle of the Veterinary Research Station, Armoedsvlakte, near Vryburg, which is situated in a dry region in the Eastern part of Bechuanaland.

An investigation was carried out on the farm by Mr. P. L. le Roux, then on the staff of this Institute. It was found that a large number of the animals were heavily infested with several species of worms, especially *Haemonchus contortus*, *Cooperia* spp., *Bunostomum phlebotomum* and *Oesophagostomum radiatum*.

Besides adult worms, the animals also harboured an appreciable number of immature individuals, showing that they were subject to a continual infection.

At the time of this investigation the climatic conditions were those of a severe drought, which is usual in that area which has a low rainfall and a long, dry winter. The temperature was fairly warm; at least sufficiently high for the development of parasitic worm larvae.

There were no moist places in the pasture or elsewhere and the drinking-water was supplied in troughs which would not allow any appreciable worm infection to occur. The origin of the constant infection at first seemed rather obscure under these conditions. Eventually the present writer suggested that the development of the larvae and the infection of the animals might take place in the following way:—

A heap of cattle dung usually dries quickly on the surface, forming a hard crust, which prevents further evaporation to a large extent. This would hold the moisture required for the development of the eggs and larvae. A further essential for development is the presence of air, which would be supplied and carried through all parts of the heap of dung through the tunnels made from the bottom by dung beetles, of which a large number of different species, large and small, can be found in every heap of cattle dung in the country. A certain amount of warmth would probably also be generated by the fermentation of the dung. If all these requirements are found in a heap of cattle dung, the latter would present the conditions of an excellent faeces culture and millions of larvae might develop in one heap from a badly infested animal.

When the larvae have reached the infective stage their migratory habits and positive heliotropism would probably cause them to wander from the heap and accumulate in the soil and grass immediately around it. Eventually the heap will become completely disintegrated and the larvae will migrate into the soil of the small area concerned. It is frequently noticed that the moisture and possibly also the warmth of a heap of dung causes the growth of a small quantity of green grass around the heap and in the site of the heap after its disintegration. Such an attractive mouthful of green grass might carry into the animal a severe infection of worms.

These suggestions appeared the more probable on account of the fact that, according to the writer's experience, cattle seem to have a very bad influence on the worm infestation of sheep, where these animals run on the same pasture. The explanation would then seem to be that the sheep, being more liable than cattle to graze off small quantities of green grass around cattle dung heaps, would acquire a heavy worm infestation through the agency of the cattle in the case of those species of worms that can be harboured by both these kinds of animals.

Preliminary tests have been carried out at Vryburg during January, 1931, and the writer wishes to acknowledge his indebtedness to Mr. J. H. R. Bisschop, who is in charge of that station and who has done a large part of the work.

Four fresh dung heaps were selected and covered with wire gauze after sufficient time had been allowed for dung beetles to enter. Specimens were taken from the second to the tenth day of the dung, the soil beneath and the soil next to the heap. These specimens were placed in the Baermann separating apparatus for 24 hours. A small quantity of water was then drawn off at the bottom, placed in tubes containing formalin and examined later.

The result showed that larvae of the worms previously mentioned developed in the dung to the infective stage within six days. It also seemed as if there was a tendency of the larvae to migrate from the heap, as had been suggested, since in most cases more larvae were found in the soil around the dung than below it. All the specimens also contained free living nematodes, usually in large numbers.

The number of parasitic nematode larvae found was never a very large one. Whether this is due to the fact that the animals concerned were not heavily infested—they had received several anthelminthic treatments—or whether development in a dung heap is not as successful as suggested, remains to be seen. The preliminary test, however, shows that development can take place under the conditions described.

A more extensive investigation on this subject is to be carried out in the early Spring of this year, under the same climatic conditions that obtained when the first investigation was made.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

A Second Species of the Nematode Genus Acanthoxyurus.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

In 1928 Sandground described, from the "flying squirrel" *Anomalurus orientalis* Peters, a worm which he named *Acanthoxyurus anomaluri*, placing it in the superfamily Oxyuroidea. Unfortunately Sandground had only female specimens and could therefore not determine the affinities of this worm more exactly. Since the female genital organs appeared to be single, Sandground thought that the worm may be allied to the Atractidae. In fact, the structure of the head resembles to some extent the head of *Labiduris*, although in the latter the two ventro-lateral lips bear special organs, while in *Acanthoxyurus* it is the dorsal side of the head which shows special structures and the oesophagus does not show the developments which are usually found in the worms grouped in the family Atractidae.

In 1927 Captain G. C. Shortridge, of the Kaffrarian Museum, Kingwilliamstown, Cape Province, kindly collected for this Laboratory some worms from mammals in the Kakaoveld, a portion of the Kalihari Desert extending into South West Africa.

This paper is based on material contained in two tubes of this collection; one marked "*Petromys cuneatus*, Katijmura, Central Kakaoveld, 20.4.27" contained two female worms and the other tube marked "*Petromyscus*, Kakaoveld" contained one female. The worms are stout and of moderate size, measuring 22-22.5 mm. in length and their maximum thickness is 1.6-1.8 mm. They taper slightly towards the anterior extremity, while the posterior portion tapers evenly to a thin tail (Fig. 1) as is usual in Oxyurid females.

The cuticle bears coarse transverse striations at intervals of about 0.04 mm.

The anterior extremity is bent slight towards the dorsal side, so that the mouth opening, although it is anterior, also points in that direction. The mouth is surrounded by three small lips, a dorsal and two subventrals. There is no buccal cavity or only a very small one, since the oesophagus begins at the bases of the lips.

The worm has a prominent mouth collar or "head," which is constricted off from the rest of the body (Fig. 2); this portion also bears transverse striations ventrally and laterally. On the dorso-lateral aspect there is on either side a bulging prominence containing a large

papilla (Figs. 2 and 3) and there are also two small, lateral papillae situated farther back on the sides of the "head." Posterior to the large dorso-lateral papillae there are another two prominences separated in the mid-dorsal line by a deep cleft, each bearing a sharp, backwardly direct "tusk," and ventro-medially to the latter a small tubercle. All these structures are situated on the "head" of the worm. The projecting portions of the "tusks" are 0.19 mm. long. Behind the "head" there are on either side two subdorsal lappets formed of folds of the cuticle, running in a longitudinal direction, the one lappet being only half as long as the other. These lappets also show transverse striations. The longest lappet measures 0.4 mm. Owing to the thickness of the worms, the internal structure is difficult to make out, and one specimen had therefore to be dissected to obtain definite knowledge on some points.

These worms do not have the large cervical glands described by Sandground in his species; in fact, no such glands could be found in the dissected worm. When viewed from the outside it may seem as if there are cervical glands, but this is the ovary which lies coiled up around the oesophagus.

The oesophagus is small in proportion to the body of the worm. It has a club-shaped anterior portion and an almost spherical bulb; the total length is 1.08 mm. and the bulb has a diameter of 0.29 mm. The anterior end of the intestine is wider than the following portion.

The nerve ring surrounds the anterior narrow portion of the oesophagus. Cervical papillae could not be found. The excretory pore is situated 1.7 mm. from the anterior extremity, behind the oesophagus, and opens into a large vesicle.

The vulva is prominent, owing to the thick walls of the vagina which project slightly, and is situated at about the middle of the worm, 11.5 mm. from the anterior extremity. The vagina leads inwards and slightly forwards; it is 0.96 mm. long and half as wide. It is followed by a voluminous ovejector, filled with eggs, which is 1 cm. long and runs forwards, almost reaching the oesophagus. There it gives off a single uterus, which coils around the oesophagus posteriorly and then proceeds backward as far as the anus and again bends forward, eventually giving off the ovary, which lies in the anterior portion of the body.

The eggs measure 0.117 mm. $\times$ 0.043 mm. They have thick shells and are markedly flattened, even slightly concave, on one side (Fig. 4) and contain a single cell. Even the eggs in the ovejector show no further development.

The tail is long and thin, measuring 2.8 mm. in length.

These worms differ in quite a number of points from the species described by Sandground, as will be evident from the following generic diagnosis given by this author for Acanthoxyurus: "Oxyuroidea; incertae sedis. Females are robust worms with the head enlarged by the inflation of the cuticle on one side (ventrally) and marked off from the rest of the body by a deep cleft on the dorsal aspect. Subterminally, the cuticle is thickened to form a cushion in which the bases of four massive spines are embedded. Indentations

of this cushion produce a series of cordons. Two leaf-like cuticular lappets project from the side of the body in the cervical region. Two elongate cervical glands are present. The buccal aperture is devoid of raised lips. A single lateral sessile papilla is present on either side of the buccal aperture. There is a distinct but shallow buccal cavity. The oesophagus is divided into an anterior club-shaped part and a posterior bulb containing a valvular apparatus. The generative organs are very massive. The vulva is at the anterior third of the body. The ovejector is muscular and long. Possibly there is only a single ovary. The animals are oviparous. The tail is acutely pointed. The host is a rodent. The male is unknown."

The "cordons" described by Sandground are not evident in his illustrations. In most important points, however, the two species agree very well and for this reason the worm here described is, at least provisionally, placed in the same genus, the name *Acanthoxyurus shortridgei* being proposed for it in honour of the collector. It is hoped that some male specimens may be found to clear up the position of the worm.

The above description of *A. shortridgei* will indicate in which way the generic diagnosis will have to be modified if this species remains in the genus.

Hosts.—*Petromys cuneatus*, *Petromyscus* sp.

Locality.—Kakaoveld, South West Africa.

Types in Onderstepoort Helminthological Collection Nos. 2308 and 2309.

REFERENCE.

SANDGROUND, J. H. (1928). Some new Cestode and Nematode Parasites from Tanganyika Territory. *Proc. Boston Soc. Nat. Hist.*, Vol. 39, No. 4, pp. 131-150, pl. 16-21.

Fig. 1.

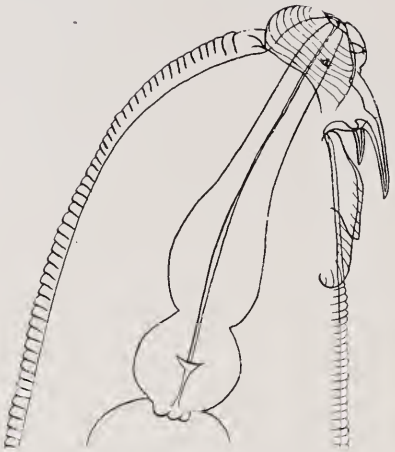

Fig. 2.

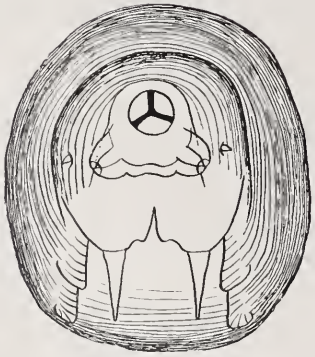

Fig. 3.

Fig. 4.

Fig. 1.—*Acanthoxyurus shortridgei*—hind end of female.
Fig. 2.—*Acanthoxyurus shortridgei*—anterior end, laterial view.
Fig. 3.—*Acanthoxyurus shortridgei*—head, anterior view.
Fig. 4.—*Acanthoxyurus shortridgei*—egg.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Two New Species of the Nematode Genus Hartertia.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

The species of the genus *Hartertia* seem to be common parasites of the bustard (*Otis* spp.). Cram (1927) has compiled records of the following: *H. obesa* Seurat, 1915, in *Otis afroides*, *O. rufieresta*.

H. confusa Cram, 1927, in *O. houbara*.

H. rotundata (Linst., 1883) in *O. afroides*, *O. macqueeni*, *O. rufieresta*.

The Onderstepoort helminthological collection contains specimens of both *H. obesa* and *H. rotundata* from *Afrotis afroides afroides* (= *Otis afroides*) and *Lophotis rufieresta* (= *Otis rufieresta*) as well as from Stanley's bustard *Neotis cafra*. These worms have been found by the writer in every bustard examined by him.

Since *Hartertia gallinarum* (Theiler, 1919) is a parasite of the fowl in South Africa and has not been found elsewhere, one must consider the possibility that this worm has been transmitted to the fowl from some wild bird which was the original host. It would thus be important to find this original host if the supposition be correct. For this reason the writer has examined the worms of bustards at all opportunities, but unfortunately such opportunities do not often arrive, since the bustard is protected by the game laws of this country and may never be shot.

In the beginning of 1930 the writer received a tube of worms collected from a bustard (species not known), collected in Zululand (Natal). The tube contained only three *Hartertia* males, of which two were alike but do not resemble any known species, while the third male also has to be described as a new species.

Hartertia zuluensis, n.p.

A single male specimen, 28 mm. long and 1.35 mm. maximum thickness. The body tapers towards both extremities. The anterior portion is curved slightly dorsalwards and the hind end is rolled in a loose spiral with the ventral surface on the inner side of the curve.

Cervical alae are not present. The cuticle has rather prominent transverse striations at intervals of 0.015-0.019 mm. There are the usual two lateral lips which are each medially divided into three

prominent lobes. The edges of the lips bear very fine denticles. Each lip bears a lateral, inconspicuous papilla and two submedian papillae. The precervical papillæ are situated very far forward in this worm (Fig. 1), on about the same level as the submedian and a short distance behind the lateral papillæ; they are acute and prominent and only 0.031 mm. from the anterior extremity in a longtitudinal direction. The head is not separated from the body in a dorso-ventral view.

The oesophagus is 4.754 mm. long and is composed of an anterior, narrow portion, 0.714 mm. long and surrounded at about its middle by the nerve ring, and a posterior, thicker portion, 4.05 mm. long.

The caudal alae are 0.965 mm. long and do not extend to the end of the tail, thus leaving a portion of the latter to project behind (Fig. 2). On the ventral surface of the alar region the hind end bears the usual cuticular bosses arranged in longitudinal rows. These bosses extend anteriorly in the mid-line as shown in Fig. 2. There are four pairs of precloacal and two pairs postcloacal stalked papillæ and five pairs of small, sessile papillae (openings of caudal glands?) near the end of the tail, arranged around a small depression.

The left spicule is slender, 2.89 mm. long, and appears to have a double barb near the end. Unfortunately the end of this spicule was somewhat masked by the gubernaculum, which resembles that of *H. rotundata* in shape. The right spicule is stouter, 0.868 mm. long and has a blunt end.

Host.—Bustard, species unknown.

Locality.—Zululand.

Type in Onderstepoort Helminthological Collection No. 2310.

Hartertia natalensis, n. sp.

Two male specimens, anterior extremity of one missing. The complete male is 54 mm. long and 1.158 mm. broad. The cuticle is more finely striated than in the previous species at intervals of 0.013 mm. The anterior end bears strong cervical alae which are 4.83 mm. long. Between the lips, dorsally and ventrally, the cuticle forms two rather prominent swellings (Fig. 3).

The cervical papillæ are situated near the bases of the lips, 0.135 mm. from the anterior end; a rather characteristic feature is that both these papillæ are placed dorsally to the beginning of the cervical alae (Fig. 3) and not in the mid-lateral line. The excretory pore and the nerve ring are both on a level of 0.675 mm. from the anterior end.

The two lateral lips are medially divided into three parts each, which show up very prominently. Each lip bears a flat lateral and two pairs of submedian papillæ, standing close together, but the anterior pair is pointed and the posterior pair flat.

The oesophagus is 7.9 mm. long and does not clearly show the usual two parts.

The caudal alae are strong and cause a spiral curvature of the hind end; they are 1.64 mm. long in both males and reach the end of the tail. The ventral surface is covered with cuticular bosses, showing the pecularity in both males, that a ring of small bosses surrounds the end of each of the papillae as shown in Fig. 4, while the rest of the bosses are arranged in longitudinal rows as usual.

There are four pairs precloacal and two pairs postcloacal pedunculated papillae and five pairs of small, sessile papillae near the tail end.

The left spicule is slender and 4.92-5.02 mm. long in the two males. It bears near the acute distal extremity a large double barb and a smaller single one. The right spicule is 0.772 and 0.926 mm. long in the two males; the shorter right spicule is in the specimen with the longer left spicule. The gubernaculum is triangular in shape.

Host.—Bustard, species unknown.

Locality.—Zululand.

Types in Onderstepoort Helminthological Collection No. 2311.

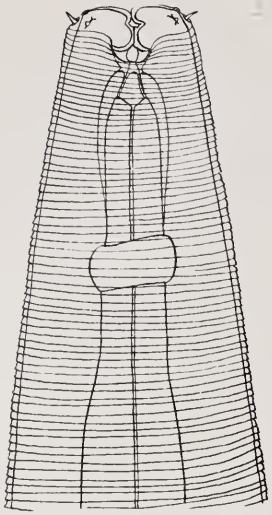

FIG. 1.

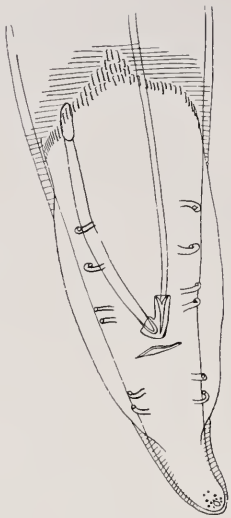

FIG. 2.

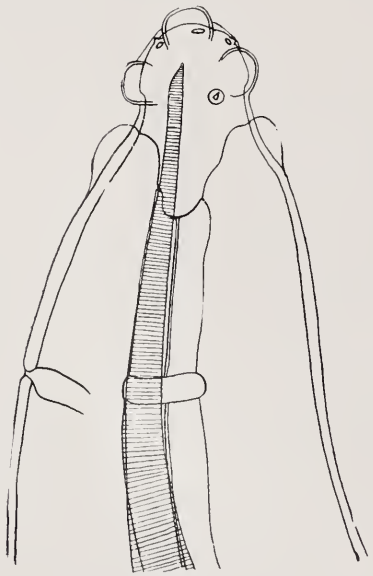

FIG. 3.

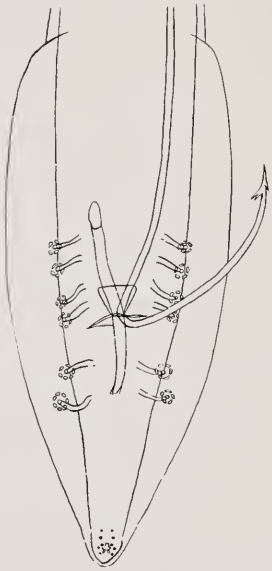

FIG. 4.

FIG. 1.—*Harterlia zuluensis*—anterior end, dorsal view.
FIG. 2.—*Harterlia zuluensis*—hind end of male.
FIG. 3.—*Harterlia natalensis*—anterior end, lateral view.
FIG. 4.—*Harterlia natalensis*—hind end of male.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Two New Nematodes from the Suricat (Viverridae).

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

The Suricat (*Suricata suricatta*) is one of the small carnivora which inhabit many of the flat open plains in various parts of South Africa and which are the chief carriers of rabies.

In connection with rabies the Suricat is not as important as other small carnivora, especially the mungoose *Cynictis penicillata*, but it has been found to carry the disease and hence it must be included where attempts are made to exterminate these small carnivora. Such a campaign is at present being attempted in the vicinity of Dealesville in the Orange Free State, and the following worms were recovered from the intestines of three Suricats sent to the writer by Mr. J. White, an assistant at this Institute, who is in charge of the operations at Dealesville. Besides the two species described, the material also contained a *Spirura* sp. from the stomach and a *Molineus* sp. from the small intestine, but owing to insufficiency of material, these are being left over until more material may be available.

Habronema whitei n. sp.

Small, delicate, white worms, tapering slightly towards both extremities. The cuticle bears fine transverse striations and there are a pair of lateral alae in both sexes, which start a short distance behind the anterior extremity and continue down the sides of the body to near the hind end. In the male they stop just before the beginning of the caudal alae. Cervical papillae are present but very asymmetrical; the left is situated just anterior to the beginning of the left lateral ala, 0.076 mm. from the anterior end in the male and 0.071 mm. to 0.098 mm. in the females, while the right is placed farther back, in the region of the nerve ring and at the side of the right lateral ala (fig. 1), 0.247 mm. from the anterior end in the male and 0.243–0.251 mm. in the females. The mouth opening is surrounded by two lateral lips, each bearing three papillae. There are no teeth, but the lips have their inner surfaces trilobed as usual. The vestibule is relatively short, measuring 0.027 mm. in both sexes.

The oesophagus has the usual two parts, the anterior, muscular portion being 0.314 mm. long in the male and 0.294 mm. in the females, the posterior glandular portion measuring 1.64 mm. and 1.61–1.65 mm. respectively.

The distances from the anterior extremity to the nerve ring are in the male 0.212 mm. and in the females 0.216–0.231 mm. and to the excretory pore in the male 0.239 mm. and in the females 0.247–0.259 mm. The excretory pore is situated sometimes in front of, but usually behind the right cervical papilla.

The male, of which there was only one specimen, is 4.6 mm. long and 0.137 mm. broad. The hind end is slightly coiled and bears caudal alae which are slightly asymmetrical, the left being 0.372 mm. long and the right 0.282 mm. The ventral surface of the hind end bears rows of cuticular bosses beginning a short distance in front of the caudal alae. There are four pairs of precloacal stalked papillae, standing in groups of two, and two pairs of postcloacal papillae, all stalked (Fig. 2). The tail is relatively short, 0.117 mm., and bears near its end on the ventral surface a number of minute papillae or pores of the caudal glands. The two spicules are slender. The left is 0.76 mm. long and has a single barb near the tip (Fig. 3); it narrows down suddenly a short distance from the end. The right spicule is simple in structure and 0.21 mm. long. The gubernaculum is somewhat V-shaped, with the left arm longer than the right, each arm guarding a spicule. A similar gubernaculum has been described for *H. chevreuxi* Seurat (1913) and *H. grimaldiae* Seurat (1915b).

The females, of which there were 9, are 5.9–6.1 mm. long and 0.157 mm. broad. The tail is acutely pointed, 0.076 mm. long, and bears at its extremity a single small appendage and a little more anteriorly a pair of ventrolateral papillae (Fig. 4). The vulva is inconspicuous, situated behind the middle of the body, 3.5–3.65 mm. from the anterior end. It leads into a short vagina with stout walls, 0.067 mm. long, which is followed by a rather roomy portion corresponding to the sphincter of other related species (Fig. 5), 0.196 mm. long. The sphincter leads into an ovejector which has a first portion with a thick cuticular lining, 0.08 mm. long and a second very muscular portion 0.165 mm. long, of which the distal half has a narrow lumen and the proximal half a wide lumen; the latter opens into two narrow tubes, of which one continues forward, while the other turns sharply backward and each opens into a uterus. The sphincter and the ovejector are directed forwards from the vagina. The uteri contain rather small eggs, measuring 0.037×0.014 mm., with thin shells and containing complete embryos.

Host.—*Suricata suricatta* (*Viverridae*).

Location.—Stomach.

Locality.—Dealesville, Orange Free State.

Types in Onderstepoort Helminthological Collection No. 2319.

This species is dedicated to Mr. White, the collector of the material.

Affinities.—Several species of *Habronema* have been described from carnivora, but they are all distinctly different from the above described species. *H. chevreuxi* Seurat (1913), from *Felis ocreata*, is nearest in size, but differs in many particulars, especially the structure of the vestibule, the female genitalia and the male spicules.

Numidica suricattae n. sp.

The material consists of a large number of specimens found in the large and small intestines of the host.

The worms are white in colour and fairly straight, tapering slightly to both ends. The cuticular striation is exceedingly fine. The mouth opening is surrounded by two indistinct lips, each bearing three papillae. The buccal capsule or vestibule is 0.063–0.067 mm. deep in the males and 0.078 mm. in the females, and is divided into an anterior and a posterior portion, the latter bearing three teeth at its base. The oesophagus projects forward between these teeth (Fig. 6). The oesophagus has a terminal bulb and is 1.35–1.39 mm. long in the males and 1.54–1.7 mm. in the females. The distances from the anterior extremity to the nerve ring and the excretory pore are respectively 0.328 mm. and 0.521–0.579 mm. in the females and 0.328 mm. and 0.428–0.501 mm. in the males.

The males are 7.7–9.1 mm. long and 0.308–0.405 mm. broad. The tail is slightly curved in towards the ventral side and has very small lateral alae near to the posterior extremity. The precloacal sucker, situated 0.72–0.75 mm. from the hind end is oblong in shape and its rim is marked by intersecting longitudinal and transverse striations of the cuticle (Fig. 7). There are ten pairs of caudal papillae, of which five are pre- and five postcloacal. As has been pointed out by Seurat (1915a) the orifices of the caudal glands may cause confusion and be counted as papillae. Baylis (1930) described *Numidica monodi* as having eleven pairs of caudal papillae, but it seems from his drawings as if he also counted the orifices of the caudal glands. In the writer's specimens the caudal glands were rather well visible (Figs. 7 and 8) and there was no difficulty in distinguishing their orifices.

As in other species of this genus the posterior lip of the cloaca is studded with small tubercles. There are two spicules, of which the right is 0.579–0.598mm. long, well chitinised and alate, while the left is 0.482–0.501 mm. long and poorly chitinised. The gubernaculum has a wide base and slender body and is 0.180 mm. long. The tail of the male is 0.231–0.270 mm. long and acute.

The females are 10.3–12.5 mm. long and 0.386–0.434 mm. broad. The tail has a mucronate tip and is 0.502–0.637 mm. long. The vulva is situated 4.8–5.9 mm. from the anterior end; in other words, slightly in front of the middle. The genital organs are as described by Seurat (1915a) for *N. numidica*, the muscular portion of the ovejector is 0.656 mm. long. The eggs measure 0.065×0.055 mm. and contain complete embryos when they are ready to be laid.

Host.—*Suricata suricatta.*

Location.—Large and small intestine.

Locality.—Dealesville, Orange Free State.

Types in Onderstepoort Helminthological Collection No. 2320.

The species of *Numidica* are all very much alike, differing chiefly in the measurements of different parts of the body. Baylis (1930) has discussed the position with regard to the genera *Numidica* and *Oxynema*. If *Oxynema crassispiculum* (Sonsino, 1889), should prove to have two spicules and a gubernaculum, the generic name *Numidica* would fall as a synonym of *Oxynema*. It seems probable that *Oxynema rectum* v. Linstow, 1899, is identical with *O. crassispiculum* and it will be noticed that the worm described above agrees very closely in its measurements with those given for *O. rectum* by von Linstow. In fact they would perhaps have to be taken as identical if the hosts and locality did not differ so much, but since such a procedure would involve the generic names mentioned, the writer would prefer to leave the matter pending until the corresponding worm from the type host of *O. rectum* should be re-studied.

SUMMARY.

Description of *Habroncum whitei* and *Numidica suricattae* from *Suricata suricatta* (Carnivora, Viverridae).

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SEURAT, L. G. (1915b). Sur deux nouveaux Spiroptères des Carnivores. *Comptes Rend. Soc. Biol.*, Vol. 78, pp. 157-161, 6 figs.

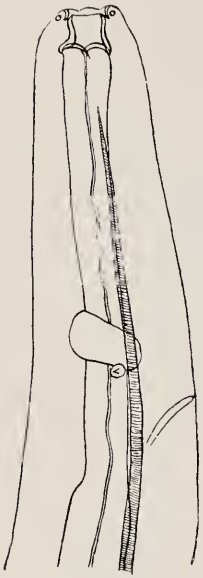

Fig. 1.

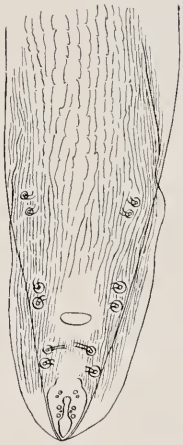

Fig. 2.

Fig. 3.

Fig. 4.

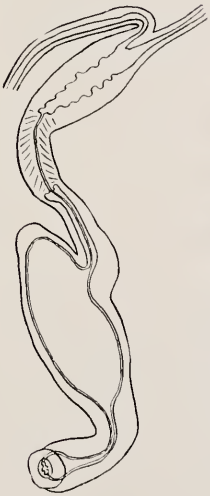

Fig. 5.

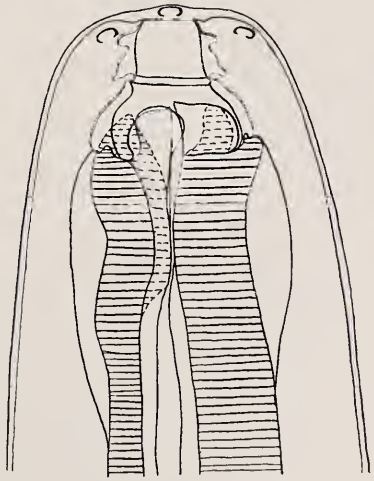

Fig. 6.

Fig. 1.—*Habronema whitei*—anterior end, lateral view.
Fig. 2.—*Hybronema whitei*—hind end of male.
Fig. 3.—*Hybronema whitei*—end of left spicule.
Fig. 4.—*Habronema whitei*—hind end of female.
Fig. 5.—*Habronema whitei*—female genitalia, distal portion.
Fig. 6.—*Numidica suricattae*—anterior extremity, lateral view.

Fig. 7.

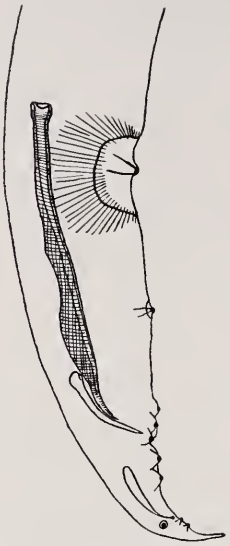

Fig. 8.

Fig. 7.—*Numidica suricattae*—hind end of male.
Fig. 8.—*Numidica suricattae*—hind end of male.

New Genera and Species of Mallophaga.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

Super-family Ischnocera Kellog.
Family Trichodectidae Burmeister.
Genus Tricholipeurus Bedford.

Thicholipeurus Bedford, Rep. Dir. Vet. Serv., Un. S. Afr., XV., p. 514 (1929).

Tricholipeurus antidorcus nov. sp.
(Figs. 1-3.)

Male.—*Head* (fig. 1) light brown, median area pale, with reddish-brown bands and mandibles. Temples with a narrow marginal band. Plate on gular region triangular. Antennae with the first segment large, very slightly longer than the second and third together; second and third segment subequal.

Thorax light brown with slightly darker lateral bands.

Pronotum with a transverse row of four minute pustulated setae in the middle; five or six on each side on the posterior margin and two on each lateral margin. Metanotum with a row of about 38 minute pustulated setae on the posterior margin. Between the fore and mid coxae there is a narrow chitinous band which widens out between the latter. In a line with the bases of the hind coxae there are two small pustulated setae in the middle.

Legs with the mid tibiae very slightly narrower and longer than either the fore or hind tibiae.

Abdomen elongated and narrow with crenulated lateral margins; pale in colour, with a transverse band and a row of minute pustulated setae on the dorsal and ventral surfaces of each segment. Tergite i with a narrow marginal band; tergites ii to vii with a short comma-shaped chitinous bar in front of each spiracle; close to these and nearer the middle there is a small ⊃-shaped chitinous spot. Spiracles median-sized. Tergite viii with a transverse row of setae in the middle, and a row on each side below. On sternites vi to vii there is a longitudinal band on each side of the genitalia. Male genitalia as in figure 2. The preputial sac (not shown in the figure) is beset with minute teeth.

Female.—*Head* as in the male, except that it is broader at the temples. First joint of antennae slightly broader than the last two, as long as the second; third joint the longest.

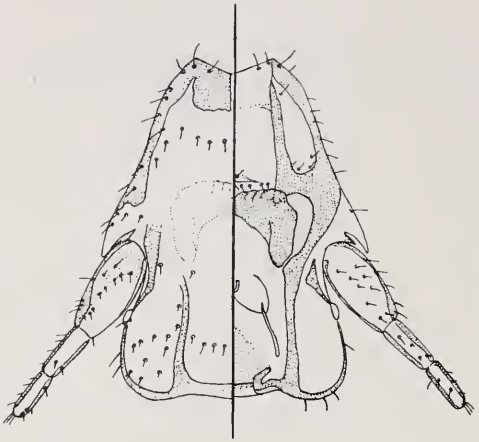

Fig. 1.

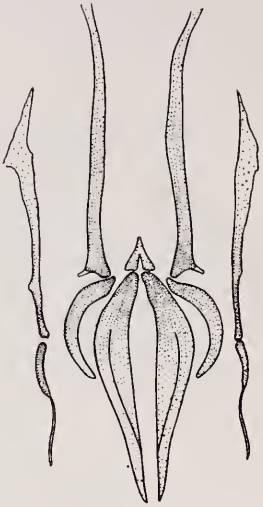

Fig. 2.

Fig. 1.—*Tricholipeurus antidorcus nov. sp.*, head of male.
C. G. Walker, del.

Fig. 2.—*Tricholipeurus antidorcus nov. sp.*, male genitalia.
C. G. Walker, del.

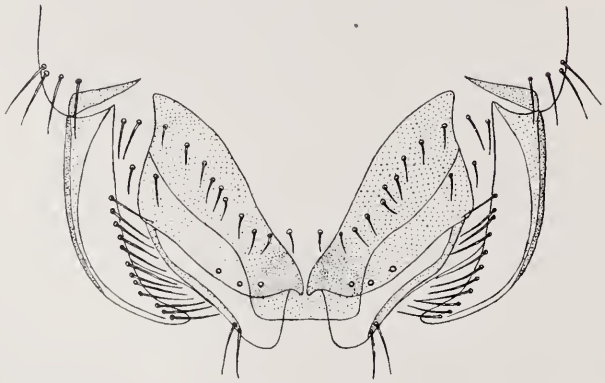

Fig. 3.—*Tricholipeurus antidorcus nov. sp.*, apical sternites of female.
G.A.H.B., del.

Thorax and *legs* as in the male.

Abdomen without ⊃-shaped chitinous spots on the tergites. Tergite viii with two short pustulated setae in the middle, and two more close together on each side between them and the lateral margin. Apical segment bilobed, with two setae on the dorsum of each lobe and two on the venter. Venter of apical sternites as shown in figure 3.

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	0.54	0.44	0.53	0.35
Prothorax	0.09	0.32	0.09	0.25
Metathorax	0.16	0.39	0.16	0.3
Abdomen	1.23	0.56	1.18	0.43
Total	2.02 mm.		1.96 mm.	

Holotype.—A male.

Described from males and females taken by the writer off *Antidorcas marsupialis* Zimm. (springbok) at Onderstepoort on the 25th July, 1930. This species is closely related to *T. oepycerus* Bedf. and *T. lerouxi* Bedf. From both these species the females can be distinguished by the apical sternites and the males by the genitalia. The female of *T. lerouxi* also has the forehead more deeply emarginated in front.

Family Philopteridae Burmeister.

Genus Otidoecus nov.

This genus is established for the reception of a new species found on *Choriotis kori* Burch. (giant bustard) at Kwambonambi, Zululand, on the 20th March, 1921.

Head almost as long as wide; forehead rounded, wider behind than in front, with a marginal band. Trabeculae very small. Antennae 5-jointed, similar in both sexes, except that the first segment of the male is slightly larger. Eyes present. Mandibles situated in a line with the antennae. Oesophageal sclerite and glands present. Temples rounded.

Prothorax and *pterothorax* wider than long, with the lateral margins rounded.

Abdomen oval with tergal plates, these being interrupted in the middle by a narrow clear space on some of the anterior segments; these plates are duplicated on some of the median segments in a similar manner to those of *Neophilopterus*, and in the male there is a median plate beneath the transverse plates on the median segments. Spiracles present on segments ii to vii. Male genitalia with the basal plate fairly broad and of medium length.

Otidoecus dimorphus nov. sp.

(Figs. 4, 5, 7*b*.)

Female.—*Head* pale brown, except the temples, which are very slightly darker. On the venter there are three setae on each side a short distance behind the anterior margin, another one further back close to the first lateral seta and one close to the antennal sinus. Gular plate indistinct.

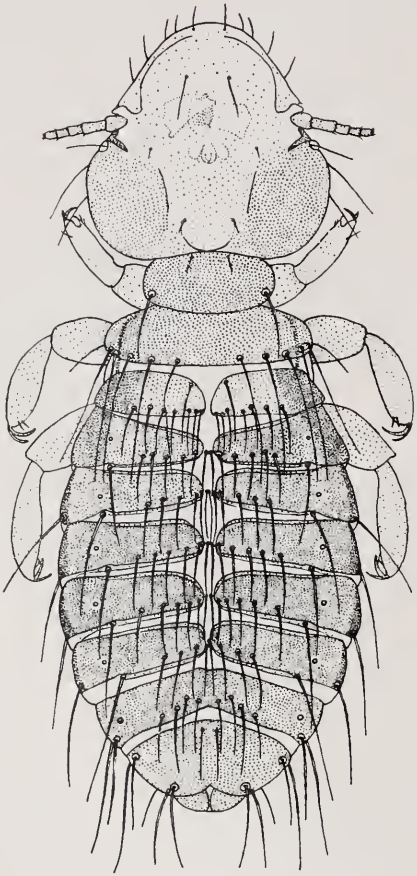

Fig. 4.

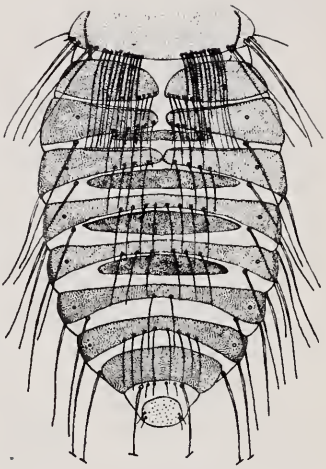

Fig. 5.

Fig. 4.—*Otidoecus dimorphus nov. sp.*, female.
Fig. 5.—*Otidoecus dimorphus nov. sp.*, metathorax and abdomen of male.
G.A.H.B., del.

Pronotum with two short setae, one above the other, on each side of the meson in front and a long one on each side behind.

Pterothorax with six long setae on each side on the posterior margin, the outer one being the shortest. On the venter there are two setae between the mid coxae and four more in a line beneath them.

Abdomen with plates and setae on the dorsum as shown in figure 4. Sternites i to v each with a narrow median transverse band and a single row of setae. Sternites vii and viii with a large brown plate, which is widest on the latter sternite; on the plate there are three pustulated setae on each side of the meson.

Male.—*Head* and *prothorax* as in the female. *Pterothorax* with sixteen long setae on each side on the posterior margin.

Abdomen with the tergites as shown in figure 5. Sternites i to iv each with a narrow median transverse band and a single row of setae. Sternites v and vi with a large transverse plate. On the plate there is a single row of about seven pustulated setae situated a short distance from the posterior margin. Joining this plate there is a slightly paler triangular plate which extends backwards to the base of the apical segment; on this plate there are two pustulated setae, one above the other, on each side. On the eighth sternite there are two setae on the posterior margin—one on each side of the plate, and on the ninth sternite there are two long setae in front, four short ones in a row in the middle, and four more on each side on the posterior margin. Male genitalia as shown in figure 7*b*.

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	0.60	0.74	0.60	0.64
Prothorax	0.17	0.40	0.17	0.36
Pterothorax	0.15	0.59	0.15	0.55
Abdomen	1.36	0.91	1.1	0.76
Total	2.37 mm.		2.02 mm.	

Holotype.—A female.

Degeeriella unicolor (Nitzsch), described from specimens taken off *Otis tarda*, does not appear to be in any way related to the above species.

Genus Otilipeurus nov.

Head slightly longer than wide; forehead semi-circular, with a marginal band. Trabeculae very small. Antennae 5-jointed, the third joint of the male with an appendage. Eyes present. Mandibles situated in a line with the antennae. Oesophageal sclerite and glands present. Temples rounded. On each side of the hind head there is a pale line where the occipital bands are usually situated.

Prothorax wider than long with the lateral margins rounded. *Pterothorax* with the lateral margins divergent; posterior margin straight, or almost so, pointed in the middle.

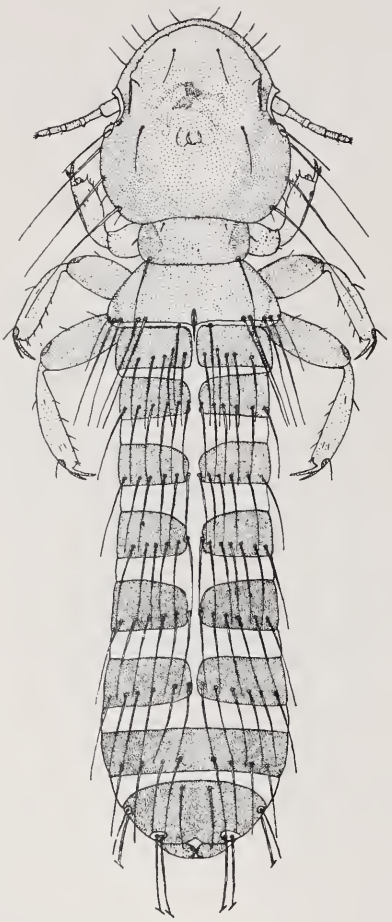

Fig. 6.

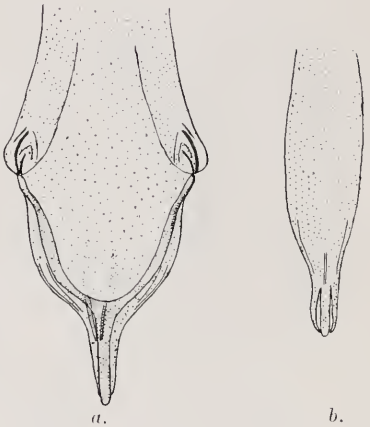

Fig. 7.

Fig. 6.—*Otilipeurus kori nov. sp.*, female.

Fig. 7.—Male genitalia of (*a*) *Otilipeurus kori nov. sp.*, and (*b*) *Otidoecus dimorphus nov. sp.* G.A.H.B., del.

Abdomen elongated, widest at either the fifth or sixth segment, with tergal plates; these being divided in the middle as a rule, but are usually complete on the posterior segments, and may be entirely so, except on the first two in the male. Spiracles present on segments ii to vii. Male genitalia with the basal plate very short and very broad.

Species found on Otidae.

Genotype.—Esthiopterum turmale (Nitzsch.).

Esthiopterum antilogum (Nitzsch.) and the new species described below must also be included in this genus.

This genus is closely allied to the preceding genus, from which it can be distinguished by the presence of an appendage on the third antennal segment in the male, the shape of the pterothorax and abdomen, the plates on the tergites, and the male genitalia. *Otilipeurus* is more closely allied to *Lipeurus* than to *Esthiopterum*, and both *O. turmalis* and *O. antilogus* should not have been transferred to the latter genus.

Otilipeurus kori nov. sp.

(Figs. 6, 7*a*.)

Female.—*Head* brown; the antennal band surrounding the forehead; the temples and a small area on each side close to the bases of the antennae slightly darker than the rest of the head. On the venter there are three setae on each side a short distance behind the anterior margin, another one further back close to the first lateral seta and one close to the antennal sinus. Plate on gular region absent.

Pronotum with a short seta on each side of the meson in front, another one on each lateral margin, and a long one on each side behind. *Pterothorax* with five long setae on each side on the posterior margin.

Abdomen with plates and setae on the dorsum as shown in figure 6. Sternites i and v each with a median transverse band and a single row of setae. Sternites vi and vii with a large brown plate, which is widest on the latter sternite and projects backwards onto the eighth sternite; the posterior margin of the plate is concave and the latero-posterior angles pointed; on the plate there are three pustulated setae on each side of the meson.

Male.—Slightly larger and paler than the female. *Head* and *prothorax* as in the female. *Pterothorax* with seven long setae on each side on the posterior margin.

Abdomen with the first two tergites similar to those of the female; on the remainder of the tergites the transverse plates are not divided in the middle; on the eighth tergite there are one long and two short setae on each side on the posterior margin instead of two long ones as in the female. Sternites i to v each with a median transverse band and a single row of setae. On the remaining sternites, except the last, there is a large, indistinct longitudinal plate. On the ninth sternite there are two short admedian setae in the middle and several short ones on the posterior margin. Male genitalia as in figure 7*a*.

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	0.64	0.59	0.64	0.57
Prothorax	0.13	0.35	0.11	0.35
Pterothorax	0.20	0.53	0.18	0.55
Abdomen	1.69	0.55	1.88	0.71 (5th segment)
Total	2.66 mm.		2.81 mm.	

Holotype.—The female.

This species is described from a female and male taken off *Choriotis kori* Burch. (giant bustard) in the Rustenburg District, Transvaal, on the 15th August, 1917.

This species is closely allied to both *O. turmalis* and *O. antilogus*. From both these it can be distinguished, *inter alia*, by the transverse plates on the third to sixth tergites being complete in the male; also the females of both these species are larger than the males. The abdomen of the female from which the drawing was made was not mounted very well; it is most probably wider in the middle than shown in the figure, and resembles that of *turmalis* in shape.

Genus Falcolipeurus nov.

Species elongated, of medium size (length 3 to 5 mm.). *Head* longer than wide. Forehead either broadly or narrowly rounded in front, with four to six more or less circular incrassations on the lateral margins; clypeal signature absent. Eyes present. Hindhead only slightly wider than base of forehead. Mandibles situated between the antennae. Pharyngeal sclerite and glands well developed. On the venter of the hind head there is a longitudinal band on each side extending to the base of the mandible. Gular plate well developed. Antennae 5-jointed, normal in the female; in the male the first joint is enlarged with an appendage on the posterior margin, and the third joint is produced at its apex into a long, curved hook.

Prothorax with sides sub-parallel. *Mesothorax* fused with *metathorax*, slightly wider than prothorax. Sternal plate either absent or inconspicuous.

Legs with the mid and hind femora and tibiae elongated.

Abdomen.—Tergites i to vii with well-developed plateral plates; these being usually connected together by less developed median bands; tergite viii usually with a complete transverse plate. Majority of sternites with a small, elongate, sub-lateral plate on each side, and usually with a short median band. Sternites viii and ix of male with an elongated lateral plate on each side. Spiracles present on segments ii to vii. Male genitalia of a distant type, as shown in figures 11 to 13. Species parasitic upon Falconiformes.

Genotype.—*Esthiopterum secretarium* (Giebel).

The following is a list of species, together with their hosts, which must also be included in this genus:—

E. aetheronomum (Nitzsch)	Host:	*Sarcorhamphus gryphus.*
E. africanus nov. sp.	,,	*Pseudogyps africanus fulleborni.*
E. assessor (Giebel)	,,	*Gypagus papa.*
E. lineatus nov. sp.	,,	*Gyps coprotheres; Terathopius ecaudatus.*
E. monile (Nitzsch)	,,	*Neophron monachus.*
E. oviceps (Piaget)	,,	?
E. perspicillatum (Nitzsch)	,,	*Vultur fulvus.*
E. quadriguttatum (Giebel)	,,	*Rostrhamus hamatus.*
E. quadrioculatum (Nitzsch) ... Syn. *E. elongatum* (Piaget).	,,	*Haliaetus albicilla.*
E. quadripustulatum (Nitzsch) ...	,,	*Vultur cinereus.*
E. sulcifrons (Denny)	,,	*Aquila albicilla.*
E. ternatum (Burmeister)	,,	*Carthartes papa.*

E. macrocerum (Piaget) described from a male taken off *Milvago pezoporus* cannot be placed in this genus. It is a straggler from an ibis.

Falcolipeurus secretarius (Giebel).

Lipeurus secretarius Giebel. *Ins. Epiz.*, p. 213 (1874).

Lipeurus secretarius (Giebel) Piaget, *Les Pédiculines*, p. 292, pl. 24, f. 2 (1880).

Esthiopterum secretarium (Giebel) Harrison, *Parasit.*, IX, i, p. 141 (1916).

(Figs. 8, 11, and 14.)

This species, which occurs on *Sagittarius serpentarius* (secretary bird), can be distinguished by the shape of the head (fig. 8) and male genitalia (fig. 11).

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	0.96	0.78	0.91	0.75
Prothorax	0.28	0.61	0.26	0.61
Metathorax	0.36	1.01	0.35	0.99
Abdomen	2.64	1.20	2.64	1.01
Total	4.24 mm.		4.16 mm.	

Falcolipeurus africanus nov. sp.

(Figs. 9, 12, and 15.)

Male.—*Head* as shown in fig. 9.

Pronotum brown at the sides, pale in the middle; posterior margin slightly rounded, with a seta on each side near the latero-posterior angle. *Metanotum* with a brown plate on each side divided in

the middle by a narrow pale line; anterior third narrower than posterior two-thirds; on the posterior margin there is a short seta near the latero-posterior angle, a longer one close to it, and three long setae in a pustule slightly nearer the meson.

Abdomen.—Tergites i to v each with a dark brown chitinous plate on each side, these being joined by a median plate, and with two rows of pustulated setae, the anterior row not extending to the lateral margins; on tergite i there is an additional row of four setae in front. Tergites vi and vii with lateral plates and two rows of pustulated setae. Tergite viii with a complete transverse plate and two rows of pustulated setae, the first row consisting of four, the second of eight setae. Apical tergite bilobed, with a small elongated brown spot on each lobe.

Sternites i to vii each with a median transverse row of setae, and a small elongated brown plate on each side. Sternite viii with a patch of long setae on the inner margin of the lateral plate at its base and several in the middle of the segment. Apical sternite with several short setae, two longer ones on the posterior margin, and two more in front of them.

Female.—*Head* as in the male. Antennae with the basal and second segments sub-equal in length; third segment the longest; two apical segments sub-equal.

Thorax as in the male.

Abdomen with tergites and sternites i to vii as in the male, except that the lateral plates on tergites vi and vii are connected by median plates, and on tergite vii there is a small plate on the posterior margin of each of the lateral plates. Tergite viii with the transverse plate almost bisected in the middle by a narrow pale area. Apical tergite bilobed with a V-shaped band on each lobe.

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	1.05	0.80	0.99	0.70
Prothorax	0.29	0.66	0.29	0.59
Metathorax	0.42	1.13	0.42	0.94
Abdomen	3.25	1.41	2.88	0.91
Total	5.01 mm.		4.58 mm.	

Holotype.—A male.

Described from two males and two females taken off *Pseudogyps africanus fulleborni* Erl. (southern white-backed vulture) in the Rustenburg District, Transvaal, on the 5th December, 1916 (Coll. W. Powell). This species can be distinguished by the shape of the head and male genitalia.

Falcolipeurums lineatus nov. sp.

(Figs. 10, 13, and 16.)

Two males and two females taken off *Terathopius ecaudatus* Daud. (bateleur eagle) in the Rustenburg District, Transvaal, on the 7th August, 1917 (Coll. W. Powell); also a male and female taken

off *Gyps coprotheres* Forst. (Cape vulture) in the same district on the 4th October, 1917. The specimens taken off the latter host were probably stragglers. We have seen a female from a *G. coprotheres*, which is very similar to *F. africanus*.

This species can be distinguished from both *F. secretarius* and *F. africanus* by the following characters:—

1. The shape of the head (fig. 10).
2. The lateral plates on the apical sternites of the male (fig. 13).
3. The absence in the male of a patch of long setae on the eighth sternite at the base of each lateral plate. (These are not shown in figs. 11 and 12).
4. The male genitalia (fig. 13).
5. The plates on the seventh and eighth sternites of the females (fig. 16).

It closely resembles *F. quadrioculatus*, but judging by Piaget's figures of *F. elongatus*, which is a synonym of this species, appears to be distinct.

Holotype.—A male off *T. ecaudatus*. *Allotype* from same host.

Measurements.

	Female.		*Male.*	
	Length.	*Width.*	*Length.*	*Width.*
Head	0.94	0.70	0.87	0.63
Prothorax	0.26	0.75	0.26	0.57
Metathorax	0.36	0.80	0.36	0.78
Abdomen	2.83	1.06	2.83	0.84
Total	4.39 mm.		4.32 mm.	

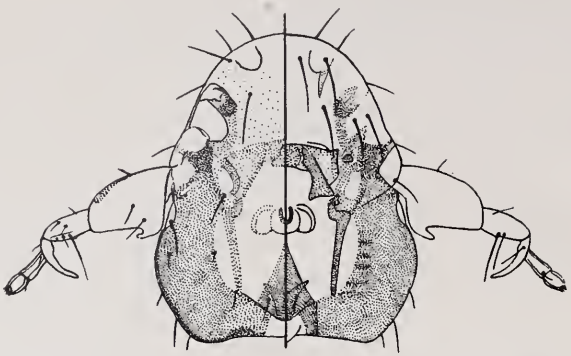

Fig. 8.—Head of *Falcolipeurus secretarius* (Giebel), male.

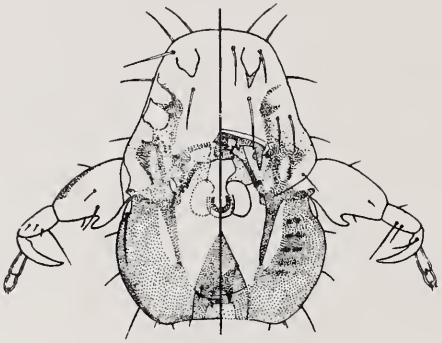

Fig. 9.—Head of *Falcolipeurus africanus nov. sp.*, male.

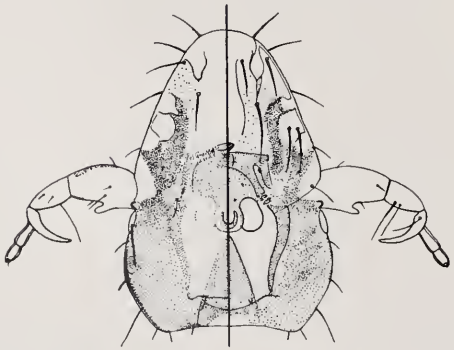

Fig. 10.—Head of *Falcolipeurus lineatus nov. sp.*, male.
G.A.H.B., del.

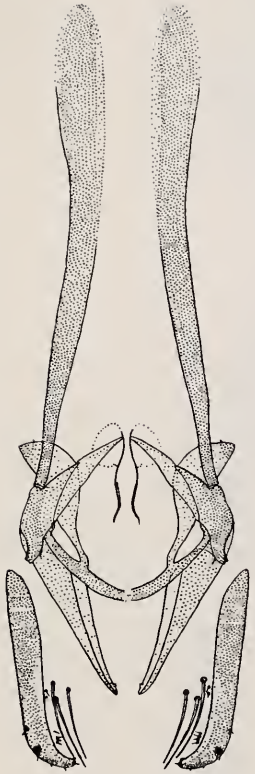

Fig. 11.

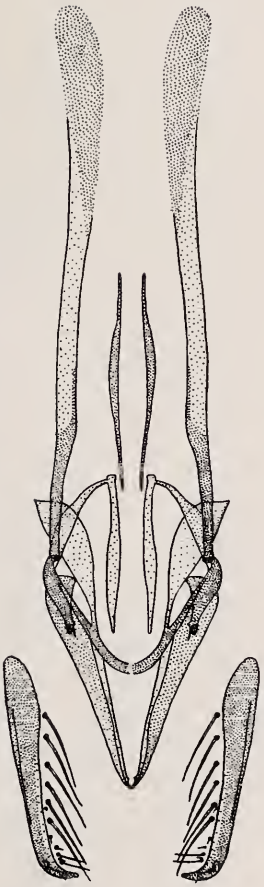

Fig. 12.

Fig. 11.—*Falcolipeurus secretarius* (Giebel), male genitalia.
Fig. 12.—*Falcolipeurus africanus nov. sp.*, male genitalia.
G.A.H.B., del.

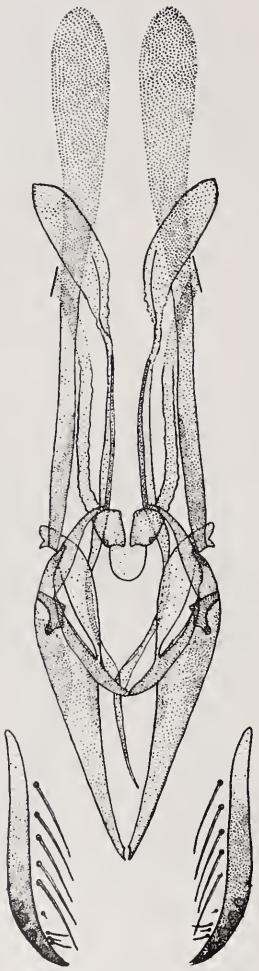

Fig. 13.—*Falcolipeurus lineatus nov. sp.*, male genitalia.

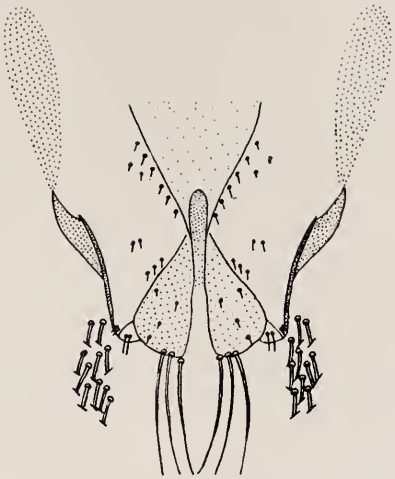

Fig. 14.—*Falcolipeurus secretarius* (Giebel), plates on apical sternites of female.

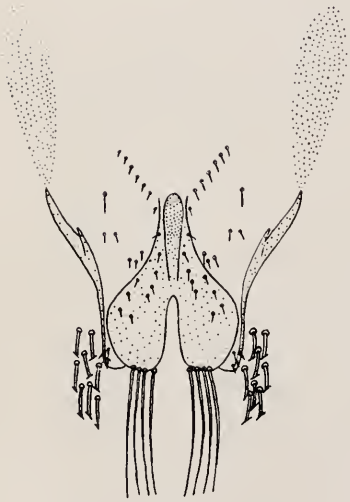

Fig. 15.

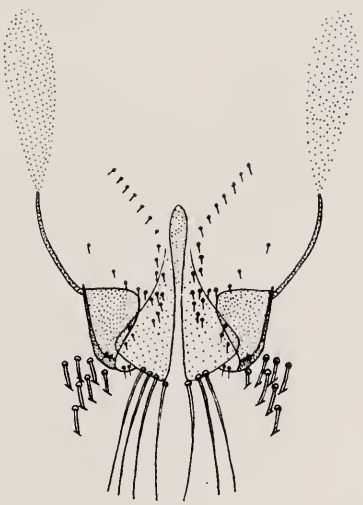

Fig. 16.

Fig. 15.—*Falcolipeurus africanus nov. sp.*, plates on apical sternites of female.

Fig. 16.—*Falcolipeurus lineatus nov. sp.*, plates on apical sternites of female. G.A.H.B., del.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

A Study of the Sheep Blow-Flies of South Africa.

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(Thesis presented to the University of South Africa for the Degree of Doctor of Science in Agriculture.)

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INTRODUCTION.

The Wool Industry of South Africa.

The production of fine wool has become one of South Africa's major industries and is, in fact, the second largest industry in the country, being only exceeded by that of gold mining. According to the latest census returns there are over forty million woolled sheep in the Union.

The quantity of wool produced in South Africa has increased enormously during recent years. In 1918 124 million pounds were produced while in 1929 the amount produced was 287 million pounds. The average production for the last five years shows an increase of 68.5 per cent. over the 1918 production.

South Africa is now the second largest producer of fine, or Merino wool in the World, Australia being the largest producer.

The average value of the wool exported from the Union during the five years 1910-1914 was four and a half million pounds per year. In 1924 the value was fifteen and three quarter million pounds and in 1927, over twenty million. In 1929 the value was fourteen and a half million but this was due to a drop in the price of wool, and not to reduced production. It will be seen at once from these figures that the importance of the wool industry to the country is enormous, and that any factor that interferes with its development is a matter of the deepest concern.

The Importance of the Sheep Blow-Flies.

The modern Merino sheep is to a marked degree subject to the attack of external parasites because of the enormous covering of wool over its whole body. Of these parasites, the sheep blow-flies are by far the worst, as their maggots feed in the wool and flesh of the living sheep and so cause the disease known as cutaneous myiasis. Moreover, the damage they cause is increasing year by year.

Such external parasites as the Ked (*Melophagus ovinus*) and the many species of ticks which attack sheep can be controlled by dipping and, being obligatory parasites, are comparatively easy to control. the blow-flies on the other hand, are not at all dependent on the sheep for their subsistence and, being possessed of prodigious powers of reproduction, their eradication is impossible and their control most difficult.

The Position with regard to Sheep Maggots in Foreign Countries.

Wherever the wool industry has developed, sheep blow-flies have become a serious menace. Maggots in the wool of sheep have been known in the British Isles for over two hundred years. To-day the position appears to be very serious indeed. At certain times of the year woolled sheep have to be examined several times a day and in warm rainy weather *L. sericata*, Meigen, females have been known to deposit their eggs on the hands of those dressing infested sheep, so strong is the tendency to oviposition in the presence of damp

wool. On the Continent the flies do not appear to be so serious a pest. Only those animals that have the wool considerably soiled with blood, faeces or urine are attacked.

Until comparatively recently the sheep blow-flies were not considered a serious pest in the United States and very little attention had been paid to them. Recently they have become much more serious and much time has been spent in studying them and devising means for their control.

Infestations occur in early spring and summer and are largely confined to lambs or heavy woolled ewes which have the wool about the buttocks soiled from diarrhoea or while lambing, and to rams at the base of the horns.

The problem appears to be complicated and made more difficult by the presence of the Screw-worm fly. *Cochliomyia macellaria*, Fab., which attacks the animals after they have become infested with the true sheep maggots and so hastens their destruction. In some years in Texas as large a proportion as one third of the rams raised on certain ranches, become infested with sheep maggots at the base of the horns. The species chiefly responsible is the black blow-fly *Phormia regina*, Meigen.

L. sericata, Meigen, is also found in North America and attacks sheep during midsummer and autumn.

The country that has suffered most severely from the attacks of sheep blow-flies is Australia, and more research work on the problem has been done there than anywhere else. A standing committee representing the veterinary, entomological, chemical and sheep and wool branches of the Agricultural Department of Australia is continuously in operation carrying out experimental work in connection with the fly. Administrative action was asked for by Australian pastoralists and cutaneous myiasis was declared to be a disease within the meaning of the Stock Disease Act of 1923. It is now compulsory for Australian farmers to dispose of carcasses.

In Australia experience has shown that as the sheep improved in woolbearing capacity it became more susceptible to the attack of the flies.

Froggott (44) in 1914 stated that the loss caused by sheep maggots in New South Wales alone was estimated at a million pounds a year and that in Queensland the loss was just as great.

There are at least five species of blow-flies which attack live sheep in Australia, only one of which is as yet found in South Africa. The species commonly found to cause cutaneous myiasis in Australia are:—

Chrysomyia rufifacies, Macqart.
Calliphora augur, Fabricius.
Calliphora stygia, Fabricius.
Microcalliphora varipes, Macqart.
Lucilia sericata, Meigen.

These are given in order of their importance in Australia. Until recently our *C. albiceps*, Wiedemann, has been confused with *C. rufifacies* which it closely resembles. It is the least harmful of the three sheep blow-flies found in South Africa.

The first cases of sheep maggots in the live wool of healthy sheep in Australia were recorded about 1904, and the pest has become steadily worse since then. Froggatt (39) in a report to the Minister of Agriculture published in the Agricultural Gazette of New South Wales first drew attention to the seriousness of the sheep maggot flies in Australia, and since that time an increasing amount of literature on the subject has emanated from that country.

The Position in South Africa.

The position in South Africa with regard to the sheep blow-fly has become very similar to that in Australia although perhaps not quite so serious as yet, because we do not run sheep in such large numbers or on such large farms. Cases of myiasis in sheep were known in South Africa as far back as 1900 but little notice was taken of the matter and it was not until recent years that the flies became a menace to sheep farming.

The reasons why the sheep blow-flies have become a more serious pest in South Africa are not far to seek. The growth of the wool industry itself is largely responsible for the damage caused by the flies. In early days little fine wool was produced and a large proportion of the sheep kept were low grade Cape sheep with very little wool on them at all. It is only in Merino sheep that cutaneous myiasis commonly occurs and with the introduction and multiplication of this type of sheep cases of the disease have become far more frequent. Moreover the Merino sheep has undergone marked changes due to continual selection within comparatively recent years. The wool has become finer and thicker, and on a good Merino sheep to-day there is hardly a part that is not covered. A few years ago it was exceptional to clip 5 lb. of wool from a sheep, but to-day the average is about 6 lb. and a good flock ewe may yield about 10 lb.

The increase of sheep in South Africa during the past few years has not kept pace with the increased production of wool showing that more wool is grown on a sheep than formerly.

It is the best sheep in the flock, judged by our present day standards, that is most often attacked. The very characters deemed most desirable, such as long, fine wool covering the entire sheep are most favourable to cutaneous myiasis.

The prevalence of maggots in Merino sheep is changing the ideals of the breeder however, and what is now sought is a plain bodied animal with as few folds as possible.

The destruction of carnivorous birds has undoubtedly had much to do with the increase of the blow-flies. In certain parts of the country vultures were previously very abundant and an animal that died was devoured before fly-maggots could develop in it. This is not the case to-day. There has been far too much indiscriminate shooting of beneficial birds in South Africa and some of those most useful in clearing the country of carrion such as the Griffin Vulture have become almost exterminated. The same applies to carnivorous animals such as the jackal and several of our wild cats.

South Africa has been a proverbially overstocked country, and the periodic droughts during which thousands of stock die are usually followed by severe losses from blow-fly. This is because the flies

breed in enormous numbers in the dead animals left on the veld. Disease, infestation by other parasites and drought go hand in hand to increase the number of blow-flies and the susceptibility of the sheep to their attack.

At certain times of the year it is necessary to keep a constant watch for sheep infested with maggots and to treat those that have been attacked immediately.

The advantage of jackal-proof fencing on which many thousands of pounds have been spent is thus largely lost, for it is no longer possible to allow sheep to graze undisturbed. In spite of constant vigilance large numbers of sheep are badly infested every season, much wool is destroyed and many sheep are killed.

From information supplied by a number of representative sheep farmers throughout South Africa, it appears that the average percentage of sheep infested with maggots during the fly season is just over ten, in spite of constant attention. Many agree that if sheep were not attended to regularly, fifty per cent. would become infested. If only five per cent. of the wool on each sheep that becomes infested during the year, is destroyed, and ten per cent. of all the sheep in the Union become infested sometime during the year, then, according to the census figures quoted above, the annual loss from this cause alone is over one hundred thousand pounds sterling. This does not take into account the value of the sheep actually killed by the maggots. In some parts of the country infested sheep are killed by them within forty-eight hours unless treated; neither does it take into account the value of the labour and materials required to prevent much greater loss.

Representatives at our agricultural congresses and members of Parliament in our House of Assembly have repeatedly asked for legislation making the control of the sheep blow-flies by farmers compulsory. This would involve the Government in considerable expense and the enforcement of such a law in our thinly populated country would present great difficulties.

The proper disposal of all carcasses on farms is compulsory by law in many other countries however, and it is not unlikely that similar legislation will be found necessary in South Africa in the near future.

The control of the pest is at present in the hands of the farmers themselves and is largely a question of co-operation amongst them. The sheep farmers are alive to the seriousness of the position and in many parts "Blow-fly eradication clubs" have been formed under the auspices of Farmers' Associations with the object of the voluntary control of the flies. The Government is doing its utmost to encourage these efforts which have been crowned with remarkable success in some areas.

Much information on the subject of sheep blow-flies has been accumulated during the present investigation, and it is the object of this paper to give this in as concise a form as possible. The methods of control which have been so successfully used throughout the country have been largely based on this information, but are not dealt with in this account. They are described in various bulletins and more popular articles which have appeared from time to time in Government publications.

The Investigation.

The present investigation was instigated by Mr. R. W. Thornton, then Principal of Grootfontein School of Agriculture, who called a committee to draw up a scheme of work in 1921. The work was not commenced until September 1922. Mr. Owen Wahl, the Entomologist of this institution had been away for a year in the United States and Australia and came fresh to the work with ideas gathered abroad. Some of the control measures used in Australia against sheep maggot flies were tested, particularly trapping and poisoning, but it was soon evident that the Australian traps and methods were not altogether suitable to South African conditions and that there was much to be learnt before the flies could be controlled here. In 1923 Mr. Wahl was transferred and the present author took over the work. The investigation has been carried on since then as the major project of the Entomological Section but owing to pressure of teaching and extension duties progress has of necessity been slow.

PART I.

STUDIES OF THE FLIES.

The Sheep Blow-Flies as Parasites.

The flies that attack sheep have been called sheep blow-flies or sheep maggot-flies to distinguish them from other blow-flies that do not lay their eggs on living sheep. In order to discover which species of flies are responsible for the sheep maggots in South Africa, large numbers of maggots were taken from live sheep all over the country and identified. The surest method of identification was to rear them in pieces of meat under large bell jars, allow them to pupate and then to identify the flies that emerged. In all cases the flies that emerged belonged to one of the three species *Chrysomyia chloropyga*, Wiedemann, *Lucilia sericata*, Meigen, or *Chrysomyia albiceps*, Wiedemann. The maggots of *C. chloropyga* and *L. sericata* were the ones most commonly found—the two species being about equally abundant, while the maggots of *C. albiceps* were much less common on sheep and usually appeared in the autumn.

The development of facultative parasitism amongst the blow-flies offers one of the most striking and interesting cases of change of habit, or variation, to be found amongst the insects.

There is no doubt that as a group the blow-flies are essentially scavengers and that the natural food of the larvae is carrion. In the case of the flesh-fly, *Sarcophaga haemorrhoidalis*, Meigen, the attraction is more marked when the meat is several days old and lasts until it has almost completely rotted and dried up.

The sheep blow-flies are very strongly attracted to fresh meat and will lay their eggs on it almost immediately it is exposed to them if weather conditions are favourable.

In the case of *L. sericata* there are records of its causing myiasis in cattle, but usually by attacking wounds that have become festered and refuse to heal. It will often lay its eggs in the eyes of unhealthy animals. *Wohlfahrtia magnifica*, Schin., has been found to attack human beings in South Africa, by ovipositing in old wounds. Porter (131). The screw-worm fly of America, *Cochliomyia macellaria*, Fab., attacks wounds on cattle and other animals, including wild animals, laying its eggs on the exposed living flesh and thereby causing myiasis which is a terrible scourge in some parts of that country. Blood seems to be particularly attractive to this fly and the maggots when once established penetrate practically sound tissue. The flies breed very readily in carcasses however.

In the case of the Indian and Central African screw-worm fly *Chrysomyia bezziana*, Villeneuve, we find that it has become entirely parasitic, the larvae living in wounds of cattle but not developing in carcasses. Patton (124.)

The steps leading to a parasitic mode of life in the blow-flies appear therefore, to be as follows:—

(1) Flies attracted to, and larvae developing in decaying meat.
(2) Flies attracted to, and larvae developing in fresh meat.
(3) Flies attracted to, and larvae developing in ulcerating wounds or dead flesh on live animals.
(4) Flies attracted to and larvae developing in sound flesh of live animals.
(5) Flies and larvae entirely dependent on living animals for their existence.

We do not have records of flies belonging to the last two groups in South Africa except *C. bezziana* in the Northern Transvaal.

When we come to consider the question of cutaneous myiasis in sheep caused by the sheep blow-flies, the case is rather different but what has just been said has a very decided bearing on the subject.

The sheep blow-flies all breed freely in carcasses, usually laying their eggs in natural openings such as the nostrils, mouth and anus. In most cases the development of the habit of attacking live, healthy sheep appears to be comparatively recent. *Lucilia sericata* has been known as a parasite of sheep in the British Isles for many years and was recorded by one Willian Ellis in a Dublin publication as early as 1749. In Australia and South Africa, however, the indigenous sheep maggot-flies seem to have acquired the habit within the last thirty years or so, there being far more temptation for them to strike live sheep now than formerly owing to the improved condition of the sheep.

The long wool about the vent easily becomes wet with urine and soiled with dung and it is this wool that is particularly attractive to the flies.

In writing on this subject in Australia, Froggatt (42) expressed the opinion that during droughts, when many dead sheep covered with soiled or tainted wool lay about the runs, the flies acquired the habit of depositing their eggs on such wool and followed it up when they found the living sheep with soiled wool on their rumps. It is

still true that the flies are more strongly attracted to an unhealthy sheep than to a sound one and still more to a sheep that is in a dying condition, but because of the long wool of the Merino, perfectly healthy sheep are often attacked.

The stages in the development of the habit of attacking live sheep appear then to be as follows: —

(1) Flies attracted to, and maggots developing in any dead sheep. Eggs laid in natural openings.
(2) Flies attracted to, and eggs deposited on the tainted wool of dead Merino sheep.
(3) Flies attracted to, and eggs laid on the wool of unhealthy or dying Merino sheep.
(4) Flies attracted to, and eggs laid on the wet or soiled wool of healthy Merino sheep.

The Classification of the South African Sheep Blow-Flies.

The classification of the Muscoid flies is most difficult and there has been more confusion in regard to it than in any other group of insects. The Calyptratae includes a series of families that are exceedingly difficult to differentiate. Comstock (21) states that no two of the authorities on this group of flies agree either as to the number of families that should be recognized or as to the limits of certain well known families. This is believed to be due to the fact that this group of flies is of recent origin and the different types included in it have not become segregated by the dropping out of intermediate forms. It must also be due to the enormous number of species of these flies. Comstock divides the Calyptrate Muscids into two super-families, the Anthomyioidea, including one family, the Anthomyiidae and the Muscoidea.

The super-family Muscoidea is divided into eight families, the best known of which are the Gastrophilidae, Oestridae, Calliphoridae, Sarcophagidae, Tachinidae and the Muscidae.

It is interesting to note that the larval food habits of these flies, although very divergent, present an unbroken series ranging gradually from one food substance to another.

The most primitive feed on dead vegetable matter and from these have evolved (1) the feeders on excrement of herbivores, (2) feeders on excrement of omnivores, (3) feeders on excrement of carnivores, (4) feeders on carrion, (5) parasites of vertebrates and (6) parasites of invertebrates including insects. Shannon (149) affirms that the Calliphoridae and Sarcophagidae, in one species or another, range through all these habits.

The Calliphoridae or blow-flies are distinguished from their near relations in the other families mentioned above by the following characteristics.

The body is metallic blue or green in colour, hence the common names blue bottle and green bottle that are applied to some species. The arista of the antenna is plumose; both the hypopleural and pteropleural bristles are present; the hindermost posthumeral bristle

is almost always more ventrad than the presutural bristle and the second ventral sclerite of the abdomen lies with its edges upon or in contact with the ventral edges of the corresponding dorsal sclerites.

As with the classification so with the naming of our blow-flies, there has been much difficulty and confusion and it is for this reason that coloured plates and technical descriptions of the South African species of sheep blow-flies are included in this article.

C. chloropyga was described by Wiedemann, 1830, as a species of the genus *Musca*. It is a very distinct species and being confined to South Africa has not received much attention or been confused with other species. There are very few references to this species in the literature.

L. sericata on the other hand is a very cosmopolitan species and there is a vast literature on this fly. It has sometimes been confused with *L. caesar*, Linne., but it is now generally so well known that there is little danger of its being wrongly determined.

In regard to *C. albiceps*, Wiedemann, however, the case is very different. In practically all the recent literature the Australian sheep maggot-fly, *C. rufifacies*, Macqart, commonly called the Hairy-maggot fly, has been referred to as *C. albiceps*. This is due to the fact that Patton (125) was able to arrange specimens of the three species *C. rufifacies*, *C. albiceps* and *Musca putoria*, Wiedemann, in a more or less continuous series showing gradual variation in certain characters, chiefly the structure of the front of the males and thus believed that they all belonged to one and the same species, *C. albiceps*. Recent researches at the Imperial Bureau of Entomology have shown, however, that *C. rufifacies* is distinct from *C. albiceps*. A large number of South African specimens, both maggots and flies, were sent to England recently for use in this work and in a letter received from Mr. Holdaway, the following paragraph is of interest:

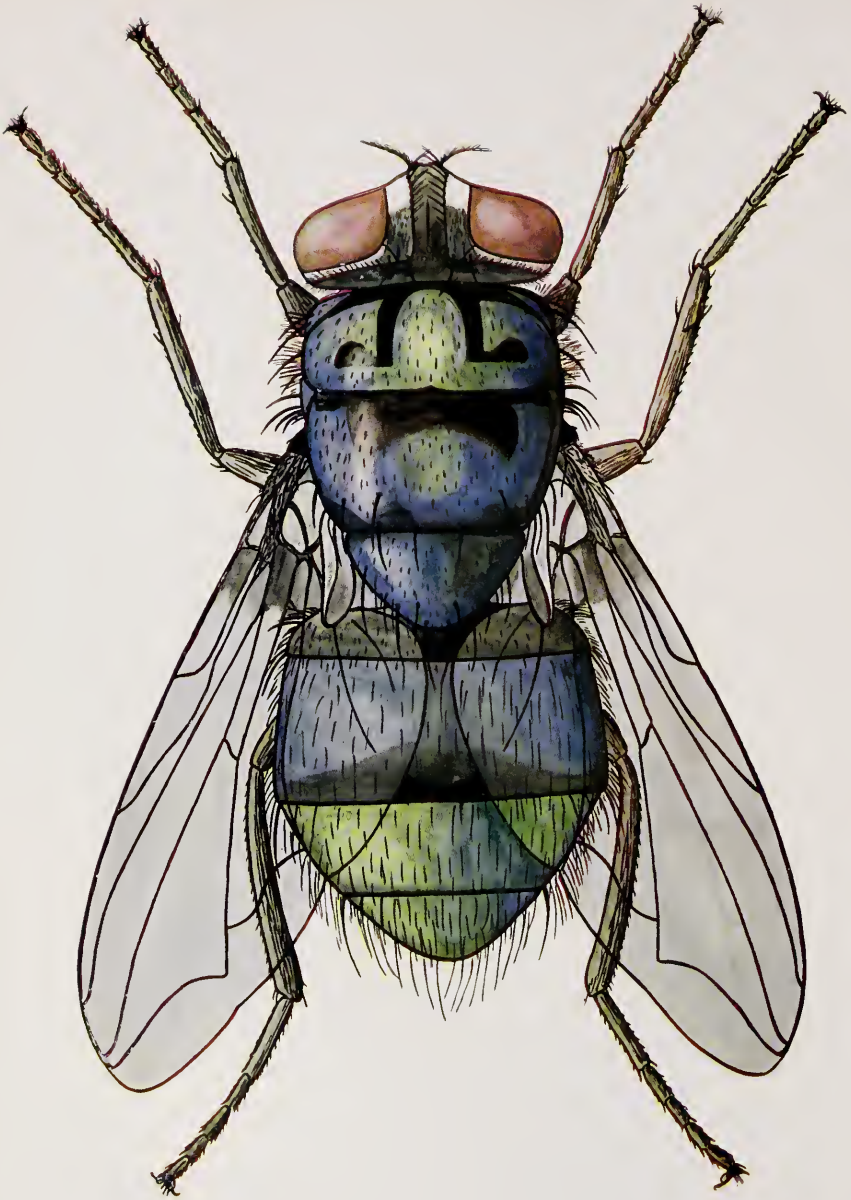
> "As far as I can see, the question of the identity of *C. rufifacies* is now fixed. I am preparing a paper on it which will deal briefly with *C. rufifacies*, *C. albiceps* and *C. putoria* from ordinary external characters as well as the structure of the genitalia. I shall also discuss briefly the synonomy which is in a terrible state."

C. albiceps was described by Wiedemann in 1819 from the Cape as *Musca albiceps*. The discovery that the Australian *C. rufifacies* is distinct from the South African *C. albiceps* is very important and means that much of the Australian work is not as applicable to our problem in South Africa as we had supposed, and that our work here is more original than we had thought.

Descriptions of the South African Species.

Chrysomyia chloropyga, Wiedemann.

In South Africa *Chrysomyia chloropyga* may be distinguished from other blow-flies by its characteristic bright green and blue markings arranged in bands across the body. For this reason it is



Chrysomyia chloropyga.

[Plate I.]

commonly called the Green-and-Blue blow-fly. It is a fairly large, thickset fly being about four-tenths of an inch in length with a large reddish-brown head. The colour of the head is due to the pigment of the large compound eyes which occupy almost its whole surface. On the prescutum is a pair of very characteristic black marks thus:— ⌋⌊ on a green background. Behind this is a band of blue and the scutellum is bluish green. The anterior half of the abdomen is blue and the hinder part bright green. The wings are clear throughout except for a slight smoky area near the base. The legs are black. (Colour Plate I.)

Technical description: Average length 8-10 m.m. Body brightly coloured green and blue and covered with a marked silvery pruinescence. Presutural area coppery green with characteristic dark markings as described above. Postsutural area black behind the suture merging into a deep purplish blue. Scutellum bluish green.

First abdominal tergite dark blue, almost black; second blue; third and fourth bright metallic green.

Face yellowish and covered with a silvery pruinescence.

In the male the head is broader than high; front very much narrowed so that the eyes almost touch; fronto-orbitals and outer vertical bristles absent. Frontal vitta at narrowest almost a line, about as wide as a parafrontal. Whole front about two-thirds the width of parafacial.

In the female the head is considerably broader than high; front not quite as broad as long however; width of frontal vitta to width of parafrontal as 4 is to 3; parafrontal about as wide as parafacial; upper half of parafrontals black. Inner and outer vertical bristles present; fronto-orbitals absent. Antennae black. Arista about as long as antenna, plumose to the tip. Third antennal joint much shorter than width of front. Border of mouth not prominent and with vibrissae considerably above it; facial ridges not ciliated; distance between these about the same as width of frontal vitta. Beard white. Palpi black with scattered black setae. (Plate IV, Figs. 1 and 2.)

Mesonotal chaetotaxy.

Presutural bristles: Acrostichals, dorsocentrals and sublaterals not developed; two humerals, one presutural, two notopleurals.

Postsutural bristles: One acrostichal, two dorsocentrals, one intra alar, two supra alars, two post alars; ten marginal and two discal scutellars.

Costal spines minute; basicosta black; subcostal sclerite black, setose; squamae white; legs black. Bright coppery green and purple of abdomen almost concealed on ventral side by a thick silvery white pruinescence. Hypopygium almost concealed. (Plate V., Fig. 1.)

Lucilia sericata, Meigen.

Lucilia sericata, commonly called the English sheep-fly, is about as long as *C. chloropyga* but is slightly more slender and is easily

distinguished from it by its bright green colour. The colour varies slightly, being sometimes almost a brassy yellow but it always has a tinge of green and the characteristic metallic lustre. The eyes are a brownish red colour and the wings are clear to the base. There are no dark bands across the body, the sutures between the segments showing only as fine lines. The legs are black. (Colour Plate II.)

Technical Description: Average length 8-10 m.m. Uniform shade of green over the body with a faint silvery pruinescence on the abdomen. Front and face more or less yellowish overlaid with silvery pruinescence. In the male the head is broader than high; front distinctly narrowed but much less so than in *L. caesar*. Fronto-orbitals and outer vertical bristles absent; frontal vitta at narrowest point, a little narrower than parafacial; parafrontal less than one half the width of parafacial.

In the female the head is very considerably broader than high; front as broad as long, sometimes broader; frontal vitta often more than twice the width of a parafrontal; parafrontal distinctly narrower than parafacial. Inner and outer vertical bristles and three fronto-orbitals present. Antennae black, arista a little longer than antenna, long, plumose; third antennal joint much shorter than width of front. Distance between facilia slightly more than the width of frontal vitta. Border of mouth not prominent and with vibrissae not much above it. Beard black. Palpi yellow with scattered black setae. Brightness of mesonotum slightly obscured by silvery pruinescence. (Plate IV, Figs. 3 and 4.)

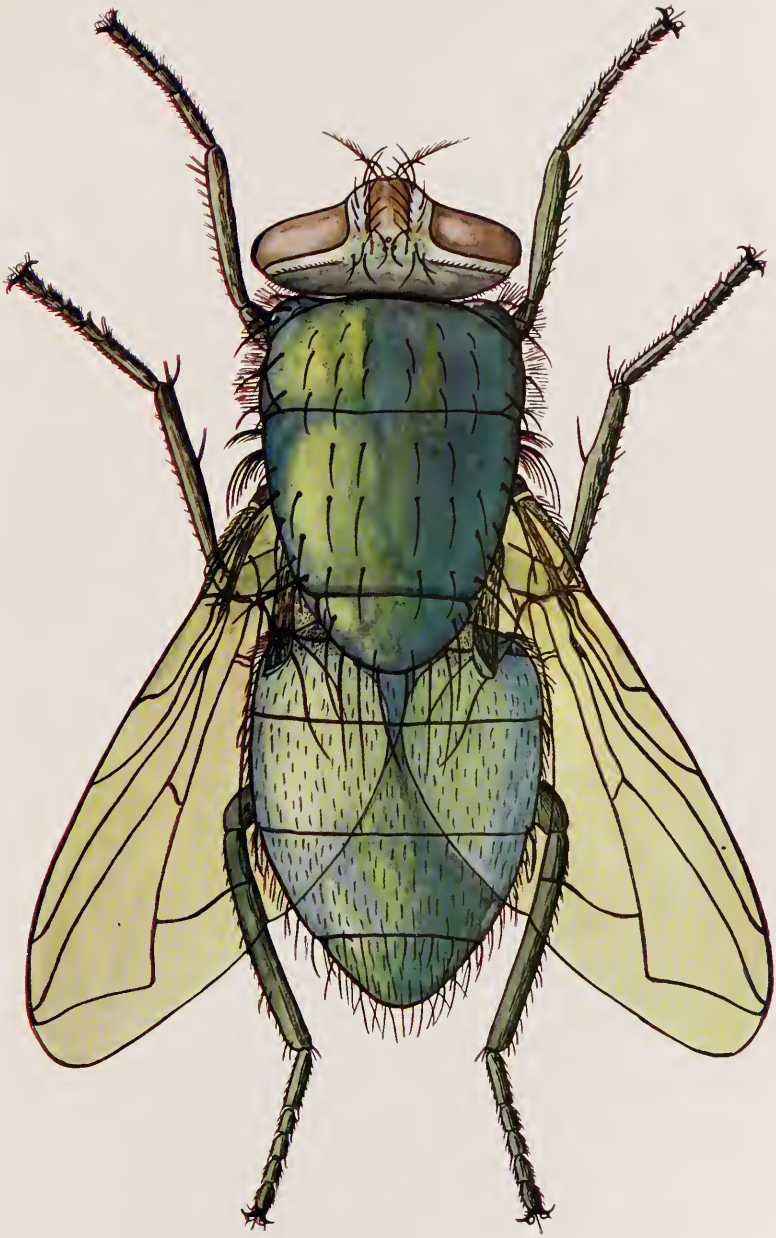
Mesonotal chaetotaxy.

Presutural bristles: Two acrostichals, three dorsocentrals, three sublaterals, four humerals, one posthumeral, one presutural, two notopleurals. Postsutural bristles: Three acrostichals, three dorsocentrals, two intra-alars, three large supra-alars, two large and one small post-alars; ten marginal and two discal scutellars. Costal spines barely distinguishable; basicosta light yellow; subcostal sclerite yellow, without setae; squamae white. Legs black.

First tergite slightly darker than the others. Rest of abdomen with coppery to greenish reflections, obscured somewhat by silvery pruinescence. Hypopygium nearly concealed; lobes of fifth sternite small, broader than long, appressed, with black setae. Outer forceps with inner surfaces facing each other, rather broad, thin, sparsely pilose; inner forceps straight, pointed, with short, loose pile. (Plate V, Fig. 2.)

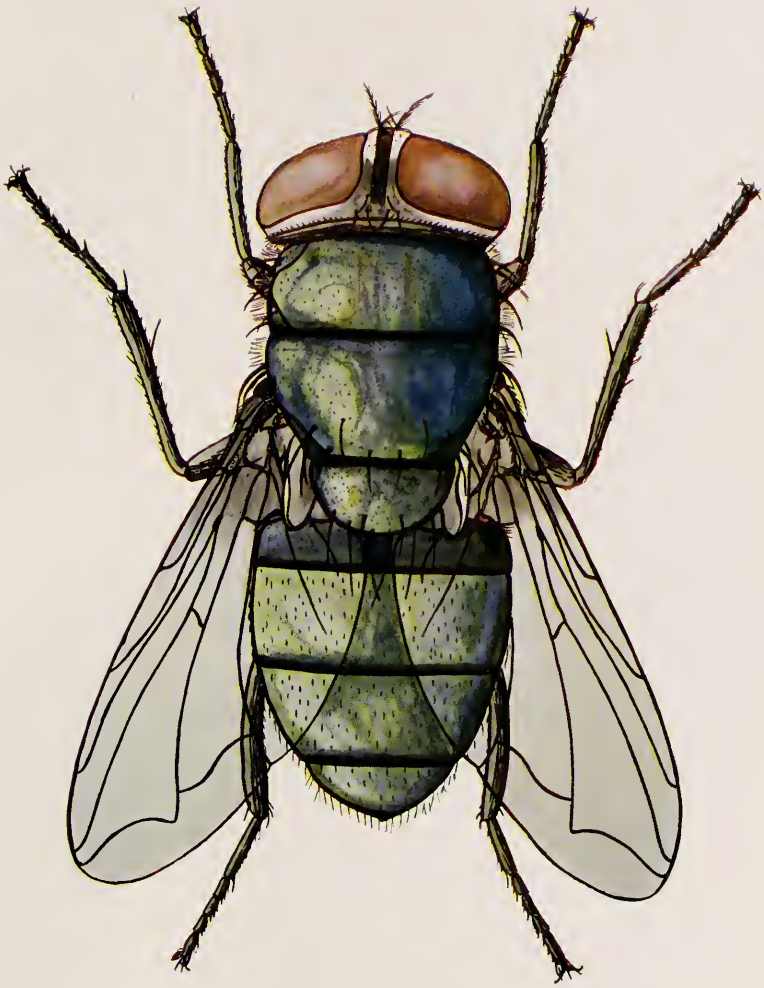
Chrysomyia albiceps, Wiedemann.

C. albiceps is commonly called the banded blow-fly. It is distinguished from the other sheep blow-flies by its green colour and the presence of dark lines or bands across its body. It is about the same size as the English sheep-fly but more the shape of *C. chloropyga*, being stouter and having the hinder end of the body less pointed. The wings when at rest also lie more parallel to, and over the body. (Colour Plate III.)



Lucilia sericata.

[Plate II.]



Chrysomyia albiceps.

[Plate III.]

Technical Description: Average length 8-10 m.m. Uniform shade of deep green over the whole body, sometimes with a deep bluish sheen in dried specimens; a very faint silvery pruinescence over the body; dark bands along the hind margins of the thoracic and abdominal segments.

Front and face yellowish, overlaid with a dense silvery pruinescence. In the male the head is broader than high; front narrowed but not as much as in the male of *C. chloropyga*; fronto-orbitals and outer vertical bristles absent; frontal vitta at narrowest part about as wide as parafacial.

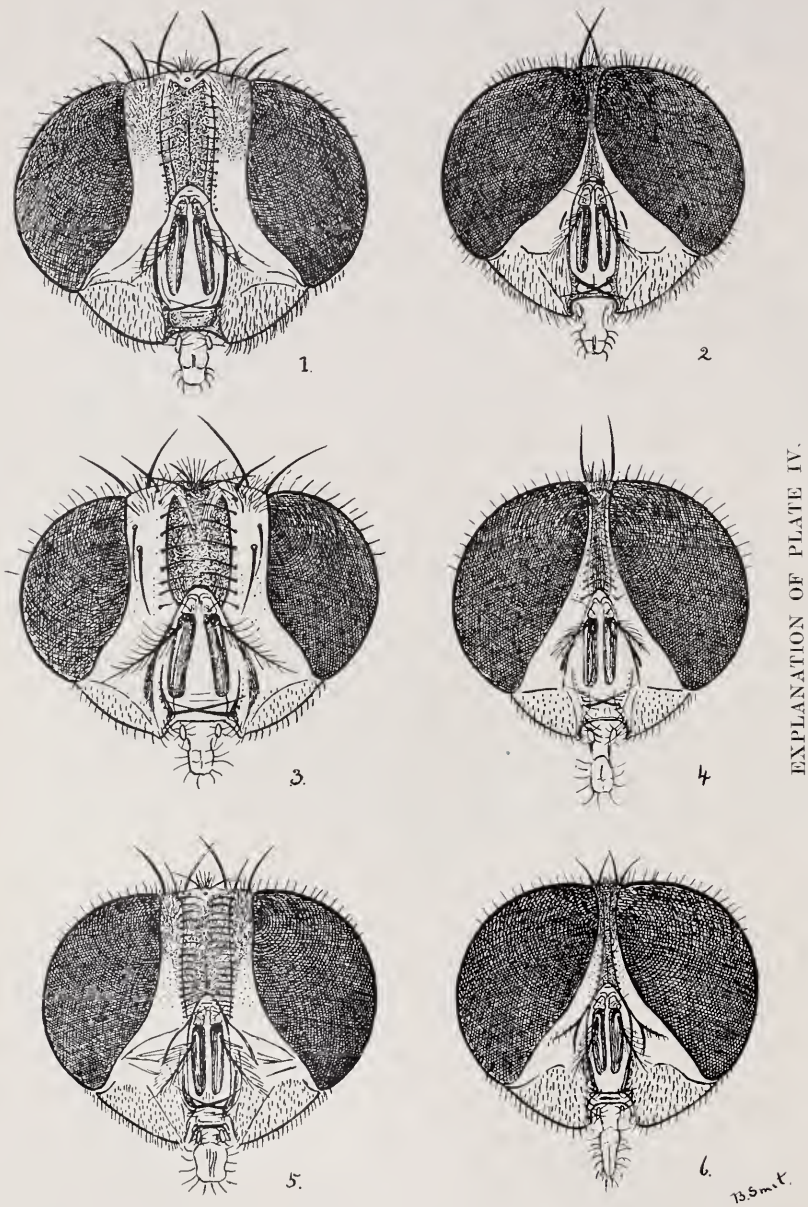
In the female the head is considerably broader than high; width of front to its height as 5 is to 6; frontal vitta about twice as wide as a parafrontal; parafrontal about two-thirds the width of parafacial; inner and outer vertical bristles present; fronto-orfitals absent. Antennae black, arista longer than antenna, plumose to the end; third antennal joint shorter than width of front; distance between facilia about the same as the width of frontal vitta; border of mouth not prominent, vibrissae considerably above it. Beard yellow, palpi yellow to orange with scattered dark yellow setae. (Plate IV, Figs. 5 and 6.)

Mesonotal chaetotaxy.

Presutural bristles: acrostichals and sublaterals not developed; one small dorsocentral, three humerals, one presutural, two noto-pleurals.

Postsutural bristles: one acrostichal, two dorsocentrals, one large intra-alar, two large supra-alars, two post-alars; ten marginal and two discal scutellars. Costal spines stouter than in *C. chloropyga*; basicosta black; subcostal sclerite black, with yellow setae. Bezzi (7) notes that the Australian species *C. rufifacies* is dinstinguished from *C. albiceps* by the presence of a strong bristle just below the prothoracic spiracle. Squamae white. Legs black. First tergite black, rest of abdomen bright green, banded as above described. Male genitalia as shown in Plate V, Fig. 3.

PLATE IV.



EXPLANATION OF PLATE IV.

Fig. 1.—*Chrysomyia chloropyga*: Head of female.
Fig. 2.—*Chrysomyia chloropyga*: Head of male.
Fig. 3.—*Lucilia sericata*: Head of female.
Fig. 4.—*Lucilia sericata*: Head of male.
Fig. 5.—*Chrysomyia albiceps*: Head of female.
Fig. 6.—*Chrysomyia albiceps*: Head of male.

PLATE V.

1

2.

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EXPLANATION OF PLATE V.

Fig. 1.—Male genitalia of *Chrysomyia chloropyga*.
Fig. 2.—Male genitalia of *Lucilia sericata*.
Fig. 3.—Male genitalia of *Chrysomyia albiceps*.

Records of Myiasis.

As mentioned above the presence of dipterous larvae in living flesh is called myiasis. When the maggots are on the surface as they usually are in the incipient stages of myiasis in sheep, the condition is described as cutaneous myiasis. The following table gives the South African records of cutaneous myiasis in sheep.

Table I.

Place.	*Month.*	*Species of Fly.*
Cape Province—		
Adelaide	May	*C. chloropyga.*
		L. sericata.
Amabele	October	*L. sericata.*
Bolotwa	November	*L. sericata.*
Dohne	May	*C. chloropyga.*
		L. sericata.
East London	—	*C. chloropyga.*
		L. sericata.
		C. albiceps.
Engwali	October	*C. chloropyga.*
		L. sericata.
Grootfontein	March	*L. sericata.*
	April	*C. chloropyga.*
		L. sericata.
		C. albiceps.
	August	*C. chloropyga.*
		L. sericata.
	September	*L. sericata.*
	October	*C. chloropyga.*
		L. sericata.
Komgha	March	*C. chloropyga,*
		L. sericata.
	April	*C. chloropyga.*
		C. albiceps.
Queenstown	April	*C. chloropyga.*
		L. sericata.
	November	*L. sericata.*
Stutterheim	May	*C. chloropyga.*
		L. sericata.
	October	*C. chloropyga.*
		L. sericata.
Swellendam	November	*L. sericata.*
Orange Free State—		
Bethlehem	—	*C. chloropyga.*
		L. sericata.
Dewetsdorp	December	*C. chloropyga.*
		C. albiceps.
Zastron	December	*C. chloropyga.*
		L. sericata.
Transvaal—		
Onderstepoort	November	*L. sericata.*
		C. albiceps.
Potchefstroom	December	*L. sericata.*
Natal—		
Dundee	November	*C. chloropyga.*
Ixopo	March	*C. chloropyga.*
		L. sericata.
York	October	*C. chloropyga.*
		L. sericata.
		C. albiceps.

In many countries myiasis is common both in domestic animals and in man. The maggots of *L. sericata* were found by Hudson (69) in the flesh of a live calf in Canada. The calf was in a sickly condition and was scouring, and the maggots were abundant around the anus and base of the tail. De Stefani (158) records that *L. sericata* causes myiasis in domestic animals in Sicily, and in South Africa the maggots of this fly were taken by Curson from the foot of a horse at Ntabanana, Zululand.

Another species of this genus, *L. argyrocephala*, Macqart, is said to attack existing sores in man, dogs, camels, and other animals in West Africa, while *L. caesar* attacks domestic animals in Sicily.

Vandenbranden (164) records the larvae of *Lucilia* from a live rat.

Our worst sheep blow-fly, *C. chloropyga* and the large blue bottle, *C. marginalis*, Wiedemann, have been found causing human myiasis in South Africa. Mr. Harris, Plant Inspector, East London, in a letter dated July 6th, 1927, describes a case as follows:—

"The District Surgeon here called me to see a case of myiasis in an old man of 76 years of age who had tramped from Graaff-Reinet. He was in a partially collapsed state when I saw him and very incoherent. From what I could gather he had first noticed an itching on his leg while on tramp but did not take much notice of it beyond scratching it. When I saw him he had three deep circular wounds just above where his boot came, and when he rolled down his sock, a mass of maggots fell away. I collected some of these and from them obtained both *C. chloropyga* and *C. marginalis.*" The flies were identified by Munro of the Division of Entomology.

It is interesting to note that Patton (124) records that *C. marganilis* will occasionally lay its eggs in, or near diseased tissues. It has not been found to attack sheep. On the other hand, there is no other record of *C. chloropyga* causing myiasis except in sheep.

C. albiceps causes myiasis only in sheep. The maggots of the fly called *C. albiceps* by Patton in India, which are believed to be *C. rufifacies*, are said to be entirely predaceous on those of other Calliphorinae. Our species which is the true *C. albiceps* shows only a tendency to be predaceous. Probably the Indian, Australian and South African species which have been called *C. albiceps* will eventually be found to be distinct.

Calliphora croceipalpis, Jaenn., is a large thickset blow-fly of a dark blackish blue colour, and is often noticed buzzing about in houses in the autumn. It has not been found to attack sheep. Porter (131) records a case of myiasis at Potchefstroom due to this fly where its maggots were taken from a patient's ear and some were obtained from the freshly passed stool of a native child in Johannesburg.

In America by far the most important myiasis-producing fly is *Cochliomyia macellaria*, Fab., the dreaded screw-worm fly of cattle. It occurs in the United States, except in the extreme north, in Mexico, the West Indies, Central America and South America. At certain seasons of the year the slightest wound is attacked, and stockmen have to be constantly on the alert to prevent heavy losses.

In some parts cattle breeding has been given up on account of this pest. The fly often attacks man and many cases of nasal and auricular myiasis have been described. Wounds in man are also attacked, and in some cases death has resulted. This species does not appear to attack healthy sheep.

In India, Burma, Assam, Ceylon and Africa, according to Patton, *Chrysomyia bezziana*, Villeneuve, is the specific myiasis-producing Calliphorine and Jack (73) calls it the "screw-worm fly of Rhodesia". It appears to be a serious pest of cattle in the Northern Transvaal where it attacks wounds. In India its larvae are commonly found in the nose and accessory sinuses, the mouth, ear, eye and vagina and in sores, cuts and abscesses, in man and animals. Patton (126) records over 170 cases of human and animal myiasis from that country due to this fly. Rouboud (138) records that this fly caused heavy losses amongst heifers at the Bouake vaccine station in French West Africa. It does not appear to attack healthy sheep and is not found in South Africa except in the Transvaal and in Zululand. It is said that this fly does not breed in meat but only in living tissue and is therefore a purely parasitic fly. This would reduce its possibilities of reproduction, and make its control easier than that of our sheep blow-flies.

In Egypt and parts of Europe a Sarcophagid, *Wohlfahrtia magnifica*, Schin., belonging to the same genus as our common Locust fly, often causes human myiasis. Its maggots were taken from the orbits and ulcers behind the ears of patients in the ophthalmic hospitals of Egypt.

Porter (131) records two cases of myiasis caused by this fly in natives in Natal. In one case in the ear and in the other in an old wound that had refused to heal. The wound contained a nest of about two hundred larvae.

Another Sarcophagid, *Sarcophaga haemorrhoidalis*, Meigen, which abounds everywhere in South Africa, has sometimes been found to cause myiasis in various animals and man, in wounds or in association with other species of flies. Its larvae have also been found in the stomach and intestines of man. Normally it breeds in decomposing meat and excrement.

Even the common house-fly, *Musca domestica*, Linne., has been found to cause myiasis. Porter records the maggots of this fly from a sore on the leg of a neglected white child living under bad conditions in a village near Durban and there are many cases of intestinal myiasis on record. Such cases must be looked upon as rather exceptional and probably due to the prevalence of the house-fly and very favourable conditions for its attack.

There are many other species of flies that have been found to attack man and animals in this way but they are too numerous to mention here. Enough has been said to show what possibilities there are in this connection. Firstly, in the way of our sheep blow-flies attacking other animals besides sheep and, secondly, of the possibility of many other flies which are not now considered as sheep blow-flies in South Africa acquiring the habit of attacking sheep. The constant danger to man himself from the attacks of these myiasis-producing flies is also apparent.

Distribution.

The distribution of each of the three South African species of sheep blow-flies will be dealt with separately.

1. *Chrysomyia chloropyga* is apparently an indigenous species. There is no record of its having been taken anywhere but in South Africa. In the Union it has been taken in traps at the following places:—

Cape Province.

Elsenburg School of Agriculture, Beaufort West, Bedford, Grootfontein School of Agriculture, Komgha, Dohne, Ida, Bolotwa, Elliot, Amabele, Stutterheim, East London, Engwali, Adelaide, Queenstown.

Orange Free State.

Dewetsdorp, Zastron, Bethlehem, Glen School of Agriculture.

Transvaal.

Potchefstroom School of Agriculture, Standerton, Amersfoort, Machadodorp, Kaapmuiden, Pietersburg, Onderstepoort, Pretoria.

Natal.

Ixopo, Dundee.

South-West Africa.

Windhoek.

Rhodesia.

Salisbury.

Most of these records were made by the author from identification of flies caught in traps operated at the respective places, and the fly has also been seen in many other parts of the Union. It is probable, therefore, that this species is well distributed over the whole southern portion of the African continent.

2. *Lucilia sericata* is a very cosmopolitan species and its distribution appears to be almost as complete as that of the common house-fly. Records of its capture all over the world are very numerous. It has been called the English sheep-fly because as stated above, it is the chief sheep maggot-fly in the British Isles and Miller (101) records it as being one of the most important sheep maggot-flies in New Zealand.

It is recorded from various parts of Europe being common in France and Germany and has been collected at Vienna and St. Petersburg and as far south as Sicily. Patton (125) records it from the North-West Frontier of India but states that he has not seen it from any other part of that country.

It is recorded from Mesopotamia and as far to the east as Yokohama in Japan. In Northern Africa it is recorded from Italian Somaliland and Tripoli.

In Australia it appears to be very common and is recorded from Queensland and New South Wales. It is also abundant in New Zealand. It is found on the Hawaiian Islands and in North America it appears to be universal. It is recorded from Ontaria, Canada, and is widely distributed in the United States, both north and south.

In South Africa the author has found specimens of *L. sericata* in traps from the following places:—

Cape Province.

Elsenburg School of Agriculture, Beaufort West, Bedford, Grootfontein School of Agriculture, Komgha, Dohne, Ida, Bolotwa, Elliot, Stutterheim, Engwali, East London, Adelaide, Queenstown,

Orange Free State.

Zastron, Bethlehem, Glen School of Agriculture.

Transvaal.

Potchefstroom School of Agriculture, Standerton, Amersfoort, Machadodorp, Kaapmuiden, Pietersburg, Onderstepoort.

Natal.

Ixopo.

Zululand.

Ntabanana.

The species is therefore probably distributed over the whole country as we should expect from its great adaptability to various climatic conditions all over the world.

3. *Chrysomyia albiceps* is probably indigenous to Southern Africa although there has been much confusion regarding its origin and distribution owing to a mistaken idea amongst writers that *C. albiceps* and *C. rufifacies* are synonyms. The two species have now been shown to be distinct.

The distribution of the true *C. albiceps* is therefore very much restricted and it will be difficult to determine it from the published records. *C. rufifacies* is restricted to the Australian-Pacific region and there is no evidence that *C. albiceps* has been found there, the many recent records of this species referring in reality to *C. rufifacies.*

Patton (125) has recorded *C. albiceps* from India, but this also is probably incorrect.

Mercier (98) discusses the distribution of *C. albiceps* and says that it has a wide distribution in the Mediterranean region, Africa, the East Indies and Australia and has been recorded from Spain and Egypt. In France it is found along the Mediterranean coast but is rare. It is very likely that he is referring to *C. rufifacies* when speaking of its distribution in the East Indies and Australia, but in Northern Africa the fly referred to is probably *C. putoria.*

The distribution of *C. albiceps* in South Africa has been studied and specimens have been taken at the following places:—

Cape Province.

Elsenburg School of Agriculture, Beaufort West, Bedford, Grootfontein School of Agriculture, Komgha, Dohne, Ida, Bolotwa, East London.

Orange Free State.

Dewetsdorp, Glen School of Agriculture.

Transvaal.

Potchefstroom School of Agriculture, Standerton, Amersfoort, Machadodorp, Kaapmuiden, Pietersburg, Onderstepoort, Pretoria.

There is no definite record of *C. albiceps* from Natal as yet.

South-West Africa.

Keetmanshoop and Windhoek.

Southern Rhodesia.

Salisbury.

This species is therefore well distributed over most of South Africa.

Localities Frequented by the South African Blow-flies.

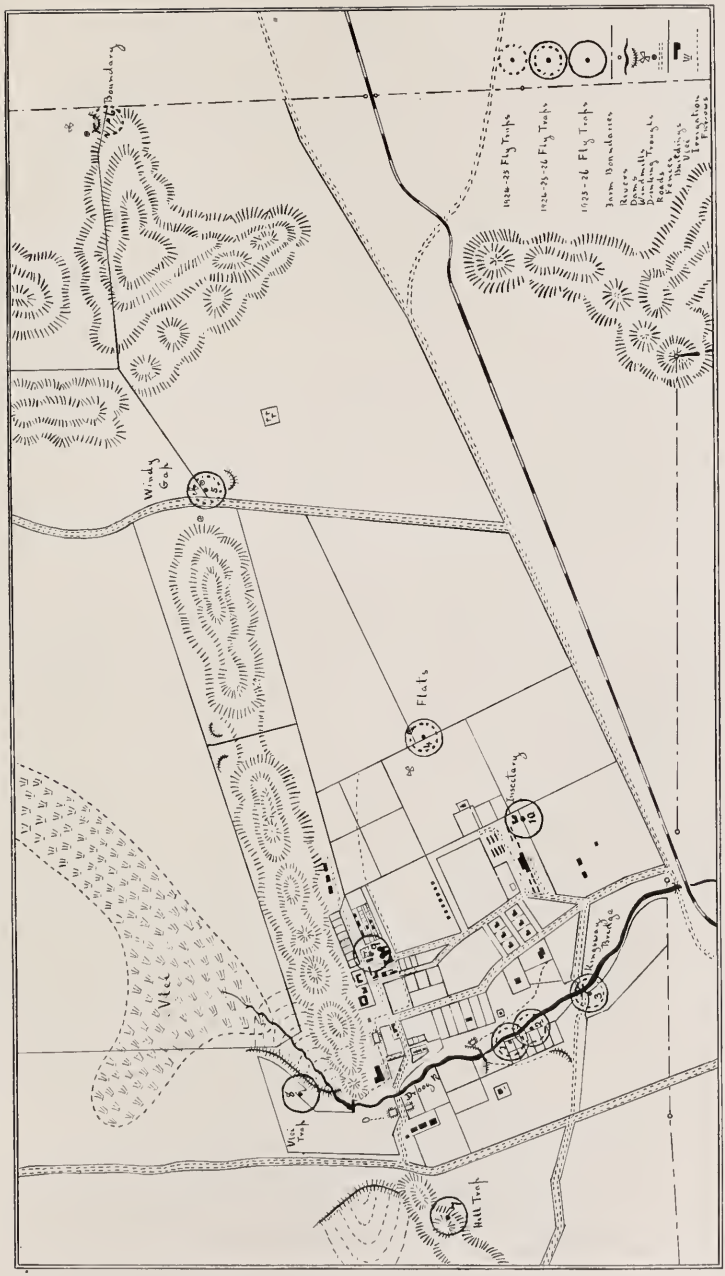
The farm of Grootfontein School of Agriculture is typical, in its topographical characters, of many sheep farms in the Cape Province, and the information gathered regarding the distribution of the blow-flies over this area will apply to any similar area in this part of the country. The farm is thirteen thousand morgen (about 28,600 acres) in extent, and is four thousand five hundred feet above sea-level. Towards the southern side of this extensive area there runs a long line of stony hills from east to west across the farm. The eastern end of this range ends near Rosmead Junction in an irregular mass of koppies separated from the main range by a gap known as Windy Gap. The height of the hills varies from 100 to 400 feet above the plain. At its western end the range is broken by a narrow gap through which the Droog River passes from what is called the "Vlei", a flat basin stretching three miles to the north, to the lower plain which slopes to the south. Across the river the range is continued, and again loses its identity in a labyrinth of steep conical hills, some of which rise to a height of 800 feet, and tops of which are the highest points on the farm. The farm buildings are situated immediately to the south of the range of hills and to the east of the Droog River and the irrigated lands border the river on both sides below the buildings, stretching a mile away from it to the east. (See accompanying photograph.) Above the gap through which the river runs are two huge storage dams, the seepage from which finds its way into the river. The river has, therefore, some water in it most of the year and its banks are bordered by poplars, willows, and reeds. In some places the reeds are very thick along the banks, affording excellent shelter for flies. About a mile from the gap below the lands

on the west side of the river are situated nine sheep paddocks, six bordering the river and three slightly removed from it. These paddocks are low-lying and often very damp; they lie on very fertile aluvial soil, are covered with thick rank grass, and are bordered by reeds which grow to the height of eight or ten feet. Below these paddocks there is a bridge called the Kingsway Bridge, which crosses the river where the banks are more open. The paddocks are used for the rearing of stud stock, and it is in these paddocks that flies are most abundant and sheep are most frequently struck.

All the roads running between staff houses and students' quarters to the east of the Kingsway Bridge and south of the lands are bordered with large eucalyptus trees. There are also numbers of pepper trees and pine trees between the buildings. The country rises to the east of the river, and stretches into a great open plain with a slight slope towards the south-west.

General view of Grootfontein School of Agriculture looking south-east from the line of hills above the college buildings. Trap No. 4 was situated in the open country to the extreme left of the picture. The sheep-sheds, where Trap No. 9 was situated, can be seen in the centre of the picture in front of the hay-stacks. Trap No. 10, near the author's insectary, was just beyond the group of buildings seen in the middle distance.

In the 1934-25 season six "Improved" blow-fly traps were placed as follows: Two in the river paddocks along the fence separating them from the river's edge. One of these was at the north end of this fence (Trap No. 1) and one about half-way down it towards the Kingsway Bridge (Trap No. 2). One trap was placed on the west side of the river, just below the Kingsway Bridge (Trap No. 3). Another trap was set in the large open plain to the south of the hills, about a mile south of them and two miles to the east of the river (Trap No. 4). This spot is beyond the lands and buildings.


Boundary
Windy Gap
Flats
Vlei
Hall Trough

It is out on the open veld, with no trees near it, but is next to one of our sheep drinking-troughs, which is supplied with water from a windmill nearby. A fifth trap was placed at Windy Gap (Trap No. 5). A sixth trap was set out near the eastern boundary of the farm, on the far side of the group of koppies, to the east of Windy Gap (Trap No. 6). This trap also stood near a sheep drinking-trough. It was about six miles in a straight line from the farm buildings.

It will be seen from the map that the latter three traps are, more or less, in a straight line running east, away from the river. They are about two, four, and six miles from the river respectively. The last two are far enough from the farm buildings to be unaffected by them. They are in typical karroo country, surrounded by low open karroo bush that does not grow to more than a foot or eighteen inches in height. There are, however, a few thorn trees within fifty yards of the trap at Windy Gap.

Tables II to VI give the numbers of the five species of blow-flies caught by each of these traps during each month of the year.

In the 1925-26 season the position of the traps was somewhat changed, and nine traps were run instead of six.

A new trap was set in the koppies to the west of the river about 300 feet above the surrounding country amongst round ironstone boulders (Trap No. 7) and also one in the "vlei" near the wall of the large storage dam to the east (Trap No. 8). A trap was also set next to the sheep-shed in the Sheep Section drafting yards (Trap No. 9), and another in one of the sheep paddocks to the south of the lands about midway between the Kingsway Bridge and Trap No. 4 (Trap No. 10). This trap was about 50 yards from the author's insectary, where there was always plenty of bad meat and smell to attract flies.

The trap near the eastern boundary of the farm was given up owing to the impossibility of attending to it regularly.

Tables VII to XI give the numbers of our five common species of blow-flies caught in these nine traps each month of the year. The traps were baited every month, and on the first day of each month they were emptied. The flies from each trap were then carefully identified and counted. Horseflesh was used as bait, with a little 1 per cent. solution of tobacco extract added to prevent maggots from developing in the bait. This smelled strongly for at least a month after setting out. In very hot weather it was found to dry up very rapidly, and, consequently, to lose its smell and attractiveness unless it was moistened with water. The direction and strength of the wind also affected the numbers of flies caught. Another difficulty was the presence of Dermestid beetles, the larvae and adults of which destroyed some of the flies after they were caught and had died in the traps. In spite of these difficulties the figures do indicate the abundance of each species at the various trapping points on the farm during each month of the year.

It will be noticed that during certain months some of the traps are recorded as having caught no flies at all. This is not strictly correct. The traps did catch a few flies during these months, but they were so few that, owing to the attack of beetles, ants, or the action of the wind, nothing was found in them but a few powdered remains.

1924-1925 *Season.*

The figures at the right of Tables II to VI giving the total number of flies of the various species caught by each trap during the year, show that in the case of the three sheep blow-flies and *C. croccipalpis* the three traps along the river caught far more flies than those farther away. Trap No. 2 caught over twice as many sheep blow-flies as either of the other river traps. This one was centrally situated in the middle of the sheep paddocks between the other two. It was nearer to the river and in a more sheltered position.

Trap No. 1 came second in the number of sheep blow-flies caught. This trap was nearer to storage dams and to standing water and was also better sheltered by reeds than No. 3, which came third in this respect.

Trap No. 4 on the open, windswept flats caught less flies than any of the others. Of the veld traps the furthest, on the boundary of the farm, caught more sheep blow-flies than the other two, the Windy Gap trap less, and the trap on the flats least. None of them caught as many sheep flow-flies as the river traps except the boundary one, which caught more *C. albiceps* than the Kingsway Bridge trap. The reason why the three veld traps caught more flies in proportion to their distance from the river is not apparent. It may be that the river itself attracted most of those flies that were near it, while the distant traps being removed from the influence of the river area formed their own centres of attraction. The seasons of maximum abundance for each of the three species of sheep blow-flies are very similar, although the flies differ greatly in their abundance. This, however, does not apply to the other blow-flies. The seasons for *C. marginalis* and *C. croceipalpis* are very different and must be dealt with separately. These flies are never as abundant as the sheep blow-flies and appear only at certain periods of the year. The trapping records of them are therefore more likely to be affected by local causes such as, for instance, the poximity of a carcass in which they would breed.

In the case of *C. marginalis* the boundary trap caught far more flies than any other. The Windy Gap trap caught more than any of the river traps, and the trap on the flats least of all. The three river traps caught a moderate number of this species. *C. marginalis* would, therefore, appear to be less strongly attracted to damp, sheltered situations than the sheep blow-flies. *C. Croceipalpis*, on the other hand, is most abundant along the river, the Kingsway Bridge trap having caught more of these flies than any other. For some reason the Windy Gap trap caught more of these flies than either of the other veld traps.

1925-1926 *Season.*

This year nine traps were operated instead of six, as explained above. (See Tables VII to XI.) They can again be divided into two groups: those along the river, including the trap in the vlei, and those away from the river. Trap No. 10 was not typical of the latter group because it was near the insectary, to which large numbers of flies were always attracted on account of the meat used in the fly cages.

In the case of the sheep blow-flies the river traps again caught far more flies than those traps away from the river except in the case of *C. albiceps*, where the Hill trap (No. 7) caught more than the " Vlei " trap or the Kingsway Bridge trap.

The trap at the sheep-sheds (Trap No. 9) caught comparatively few flies. This is not as remarkable at it might at first seem, because the sheep-yards are always kept in a clean condition and no offal is ever allowed to lie about them. It is well known that blow-flies as a general rule avoid buildings, and as sheep are not allowed to remain in the yards longer than is absolutely necessary, there is nothing to attract the flies there.

This year the Kingsway Bridge trap caught more sheep blow-flies than the others, and the other traps less in the order of their position up the river, the Vlei trap catching the least. This differs from the results of the previous season.

The trap on the flats again caught comparatively few sheep blow-flies, the Windy Gap trap more, and the trap in the hills the most of these three. It is remarkable that the trap in the hills should have caught so well. This is contrary to expectations, and there seems to be no satisfactory explanation for it.

Considering the trapping of *C. marginalis*, we find the trap near the insectary caught the most and the trap at the sheepsheds the least. The other traps all caught about the same number of flies except the Kingsway Bridge trap which caught less than the other three river traps, and the Windy Gap trap which caught less than the other traps away from the river.

In the case of *C. croceipalpis*, the Kingsway Bridge trap caught the most flies and the trap on the flats the least. The river trap caught far more flies than the others although the hill trap caught well. The vlei trap caught comparatively few.

Conclusions:—

The results of the above trapping records are somewhat confusing and no very definite conclusions can be drawn from them. One important fact seems to stand out, however, and it is that the sheep blow-flies are attracted to the river area. They are most abundant in low-lying areas where there is plenty of moisture and shelter afforded by vegetation. This we should expect, for we know that the flies must always have a supply of moisture to keep them alive.

Isolated traps seem to catch a large number of flies because they attract the flies from a large area, but on open wind-swept flats the flies are very scarce. The blow-flies are not abundant about farm buildings unless there is some definite attraction there, such as was provided by the author's insectary.

C. marginalis is not attracted by the river area but is found fairly evenly distributed over the farm, while on the other hand *C. croceipalpis* has similar habits to the sheep blow-flies, as regards its distribution.

TABLE II.

NUMBERS OF *Chrysomyia chloropyga* CAUGHT IN SIX TRAPS AT GROOTFONTEIN DURING THE SEASON 1924-25.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 1. River Trap.....	6	181	744	31,060	51,310	212	1,518	52	98	32	319	172	85,704
No. 2. River Trap.....	72	8	3,588	68,260	44,020	10,270	4,774	18	322	1,540	780	249	133,901
No. 3. Kingsway Bridge Trap..	10	74	898	26,680	51,020	51	718	37	337	3,100	442	184	83,551
No. 4. Trap on Flats.....	0	0	2	115	38	13	0	0	0	8	0	0	176
No. 5. Windy Gap Trap.....	1	3	0	230	645	0	0	0	400	570	519	47	2,415
No. 6. Boundary Trap.....	3	1	41	992	346	0	0	0	305	1,973	825	0	4,486
TOTALS FOR SIX TRAPS.....	92	267	5,273	127,337	147,379	10,546	7,010	107	1,462	7,223	2,885	652	310,233

TABLE III.

NUMBERS OF *Lucilia sericata* CAUGHT IN SIX TRAPS AT GROOTFONTEIN DURING THE SEASON 1924-25.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 1. River Trap.....	0	143	713	3,271	6,290	48	3,175	196	120	161	49	76	14,242
No. 2. River Trap.....	70	20	2,402	9,725	9,512	14,871	7,787	98	151	222	99	94	45,051
No. 3. Kingsway Bridge Trap..	6	105	769	5,223	2,700	196	1,562	98	154	1,063	79	38	11,993
No. 4. Trap on Flats.....	0	1	0	17	4	6	5	0	0	21	0	3	57
No. 5. Windy Gap Trap.....	10	43	434	251	96	0	0	0	436	418	264	20	1,972
No. 6. Boundary Trap.....	0	18	288	927	0	39	0	0	467	1,796	1,510	0	5,045
TOTALS FOR SIX TRAPS.....	86	330	4,606	19,414	18,602	15,160	12,529	392	1,328	3,681	2,001	231	78,360

TABLE IV.

NUMBERS OF *Chrysomyia albiceps* CAUGHT IN SIX TRAPS AT GROOTFONTEIN DURING THE SEASON 1924-25.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 1. River Trap.....	0	0	0	48	592	308	4,571	280	52	18	44	13	5,926
No. 2. River Trap.....	12	2	0	140	1,446	2,302	793	129	460	751	129	27	6,191
No. 3. Kingsway Bridge Trap..	0	0	0	0	88	0	1,580	106	182	1,550	100	21	3,627
No. 4. Trap on Flats.....	0	0	0	0	2	5	0	0	0	10	0	2	19
No. 5. Windy Gap Trap.....	0	0	0	0	10	0	0	0	1,144	872	151	13	2,190
No. 6. Boundary Trap.....	5	0	0	0	51	42	0	0	1,116	2,300	521	0	4,035
TOTALS FOR SIX TRAPS.....	17	2	0	188	2,189	6,657	6,944	515	2,954	5,501	945	76	21,988

TABLE V.

NUMBERS OF *Chrysomyia marginalis* CAUGHT IN SIX TRAPS AT GROOTFONTEIN DURING THE SEASON 1924-25.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 1. River Trap.....	0	0	0	0	0	0	1,126	3	76	106	60	6	1,377
No. 2. River Trap.....	0	0	0	0	0	18	497	32	284	330	41	3	1,205
No. 3. Kingsway Bridge Trap..	0	0	0	0	0	10	431	72	179	718	36	7	1,453
No. 4. Trap on Flats.....	0	0	0	0	3	0	0	0	0	14	0	6	23
No. 5. Windy Gap Trap.....	0	0	0	0	0	0	0	0	1,441	1,202	113	8	2,764
No. 6. Boundary Trap.....	0	0	0	0	4	8	0	0	6,358	3,321	118	0	9,809
TOTALS FOR SIX TRAPS.....	0	0	0	0	7	36	2,054	107	8,338	5,691	368	30	16,631

TABLE VI.

NUMBERS OF *Calliphora croceipalpis* CAUGHT IN SIX TRAPS AT GROOTFONTEIN DURING THE SEASON 1924-25.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 1. River Trap	5	89	133	32	0	0	0	0	0	0	20	55	334
No. 2. River Trap	24	0	0	94	32	0	0	0	0	17	89	101	357
No. 3. Kingsway Bridge Trap	24	90	50	351	293	0	0	0	0	27	60	88	983
No. 4. Trap on Flats	0	1	0	0	0	0	0	0	0	0	0	0	1
No. 5. Windy Gap Trap	2	18	28	0	0	0	0	0	0	29	149	17	243
No. 6. Boundary Trap	0	2	7	0	0	0	0	0	0	14	21	0	44
TOTALS FOR SIX TRAPS	55	200	218	477	325	0	0	0	0	87	339	261	1,962

TABLE VII.

NUMBERS OF *Chrysomyia chloropyga* CAUGHT IN NINE TRAPS AT GROOTFONTEIN DURING THE SEASON 1925-26.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 8. Vlei Trap	12	2	2,879	24,655	26,847	6,444	1,061	32	0	96	4,565	28	66,621
No. 1. River Trap	260	137	14,894	51,408	17,946	22,724	360	95	498	90	2,087	0	110,499
No. 2. River Trap	192	94	12,970	58,884	43,494	1,123	0	12	3,041	112	4,913	0	124,835
No. 3. Kingsway Bridge Trap	433	102	5,314	114,480	18,449	16,414	1,360	203	894	78	1,935	0	159,562
No. 9. Sheep Drafting Yards Trap	282	0	264	1,709	242	0	0	18	4	1	0	0	2,520
No. 10. Trap south of lands near Insectary	118	44	2,420	26,960	38,174	12,651	1,062	15	2,496	16	0	0	83,956
No. 4. Trap on Flats	27	2	354	12,853	650	1,376	0	6	3	450	0	0	15,721
No. 5. Windy Gap Trap	232	3	565	19,411	10,740	6,689	0	22	5	315	806	0	38,788
No. 7. Hill Trap	64	3	1,668	21,661	14,278	17,128	36	3	4	145	0	0	54,990
TOTALS FOR NINE TRAPS	1,620	387	41,228	332,021	170,820	84,549	3,879	406	6,945	1,303	14,306	28	657,492

TABLE VIII.

NUMBERS OF *Lucilia sericata* CAUGHT IN NINE TRAPS AT GROOTFONTEIN DURING THE SEASON 1925-26.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 8. Vlei Trap	19	25	1,020	5,059	7,159	1,395	52	5	3	2,965	628	328	18,658
No. 1. River Trap	59	234	3,241	6,939	3,765	4,155	126	116	40	774	499	0	19,947
No. 2. River Trap	90	158	1,970	7,420	8,527	7,647	0	586	639	202	721	0	27,960
No. 3. Kingsway Bridge Trap	33	97	1,487	14,429	4,986	2,098	83	108	689	51	298	0	24,359
No. 9. Sheep Drafting Yards Trap	78	10	179	326	99	0	0	37	0	5	0	0	734
No. 10. Trap South of Lands	64	176	0	3,343	7,258	2,033	10	1,286	1,144	0	0	0	15,314
No. 4. Trap on Flats	34	3	269	3,635	335	359	0	28	236	386	963	0	6,248
No. 5. Windy Gap Trap	79	50	380	4,029	2,349	906	0	15	2	21	293	0	8,124
No. 7. Hill Trap	3	0	357	2,006	1,323	5,281	52	2	10	55	817	0	9,906
TOTALS FOR NINE TRAPS	459	753	8,903	47,186	35,801	23,874	323	2,183	2,763	4,458	4,219	328	131,250

TABLE IX.

NUMBERS OF *Chrysomyia albiceps* CAUGHT IN NINE TRAPS AT GROOTFONTEIN DURING THE SEASON 1925-26.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 8. Vlei Trap	20	6	0	0	986	824	10	15	63	981	2,492	1,764	7,161
No. 1. River Trap	54	117	0	0	519	6,456	18	2,168	1,250	2,082	1,406	0	14,070
No. 2. River Trap	77	16	0	28	1,040	1,209	0	1,024	84	3,770	2,555	0	9,803
No. 3 Kingsway Bridge Trap	118	15	0	50	1,053	792	68	482	321	1,819	924	0	5,642
No. 9. Sheep Drafting Yards Trap	452	6	0	18	21	0	0	200	21	61	0	0	779
No. 10. Trap South of Lands	48	2	0	0	2,765	463	122	420	1,152	0	0	0	4,972
No. 4. Trap on Flats	73	4	0	0	61	1,402	0	14	681	978	1,056	0	4,269
No. 5. Windy Gap Trap	319	3	0	0	436	234	0	46	96	1,362	1,297	0	3,793
No. 7. Hill Trap	138	20	0	0	1,923	546	10	650	56	4,763	1,174	0	9,280
TOTALS FOR NINE TRAPS	1,299	189	0	96	8,804	11,926	228	5,019	3,724	15,816	10,904	1,764	59,769

TABLE X.

NUMBERS OF *Chrysomyia marginalis* CAUGHT IN NINE TRAPS AT GROOTFONTEIN DURING THE SEASON 1925-26.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 8. Vlei Trap.....	2	0	0	13	0	13	0	0	0	696	1,503	284	2,511
No. 1. River Trap.....	17	48	12	0	9	479	0	204	77	1,257	770	0	2,873
No. 2. River Trap.....	3	2	0	0	0	0	0	63	73	1,836	445	0	2,422
No. 3. Kingsway Bridge Trap..	5	2	0	0	2	43	2	58	33	655	765	0	1,565
No. 9. Sheep Drafting Yards Trap.....	16	0	0	1	0	0	0	2	0	1	0	0	20
No. 10. Trap South of Lands...	6	3	0	0	5	0	3	100	3,752	100	0	0	3,969
No. 4. Trap on Flats.....	10	0	0	0	0	1,356	0	0	96	250	450	0	2,162
No. 5. Windy Gap Trap.....	87	0	0	26	0	22	0	0	0	976	261	0	1,372
No. 7. Hill Trap.....	21	6	0	0	10	0	6	22	0	1,460	1,134	0	2,659
TOTALS FOR NINE TRAPS....	167	61	12	40	26	1,913	11	449	4,031	7,231	5,328	284	19,553

TABLE XI.

NUMBERS OF *Callipiora croceipalpis* CAUGHT IN NINE TRAPS AT GROOTFONTEIN DURING THE SEASON 1925-26.

No. of Trap.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	Totals for Year.
No. 8. Vlei Trap.....	17	9	58	13	0	0	0	0	0	5	19	68	189
No. 1. River Trap.....	34	84	222	54	0	0	0	0	26	0	4	0	424
No. 2. River Trap.....	45	65	364	0	0	0	0	0	65	0	18	0	557
No. 3. Kingsway Bridge Trap..	32	181	394	0	0	0	10	1	139	0	15	0	772
No. 9. Sheep Drafting Yards Trap.....	12	2	18	0	0	0	0	0	0	0	0	0	32
No. 10. Trap South of Lands...	10	54	109	32	0	0	0	0	0	0	0	0	205
No. 4. Trap on Flats.....	1	0	3	0	0	0	0	0	0	0	0	0	4
No. 5. Windy Gap Trap.....	85	94	40	26	0	0	0	0	10	11	8	0	274
No. 7. Hill Trap.....	27	93	300	56	41	0	0	0	0	5	126	0	648
TOTALS FOR NINE TRAPS....	263	582	1,508	181	41	0	10	1	240	21	190	68	3,105

Relative Abundance of the Various Species.

During the twelve months from July, 1924, to June, 1925, the six traps above-mentioned caught an enormous number of flies, as did the nine traps during the period July, 1925, to June, 1926.

The numbers of the various species and percentage of each of the total are given in Table XII.

TABLE XII.

Season 1924-25—Six Traps.

Species.	*Number.*	*Percentage.*
C. chloropyga	310,233	72.3
L. sericata	78,360	18.3
C. albiceps	21,988	5.1
C. marginalis	16,631	3.9
C. croceipalpis	1,962	.4
	429,174	100.0

Season 1925-26—Nine Traps.

Species.	*Number.*	*Percentage.*
C. chloropyga	657,492	75.5
L. sericata	131,250	15.1
C. albiceps	59,769	6.9
C. marginalis	19,553	2.2
C. croceipalpis	3,105	.3
	871,169	100.0

It is interesting to note that, of all the blow-flies caught during the first year, 95.7 per cent. were sheep blow-flies and during the second year the percentage was 97.5.

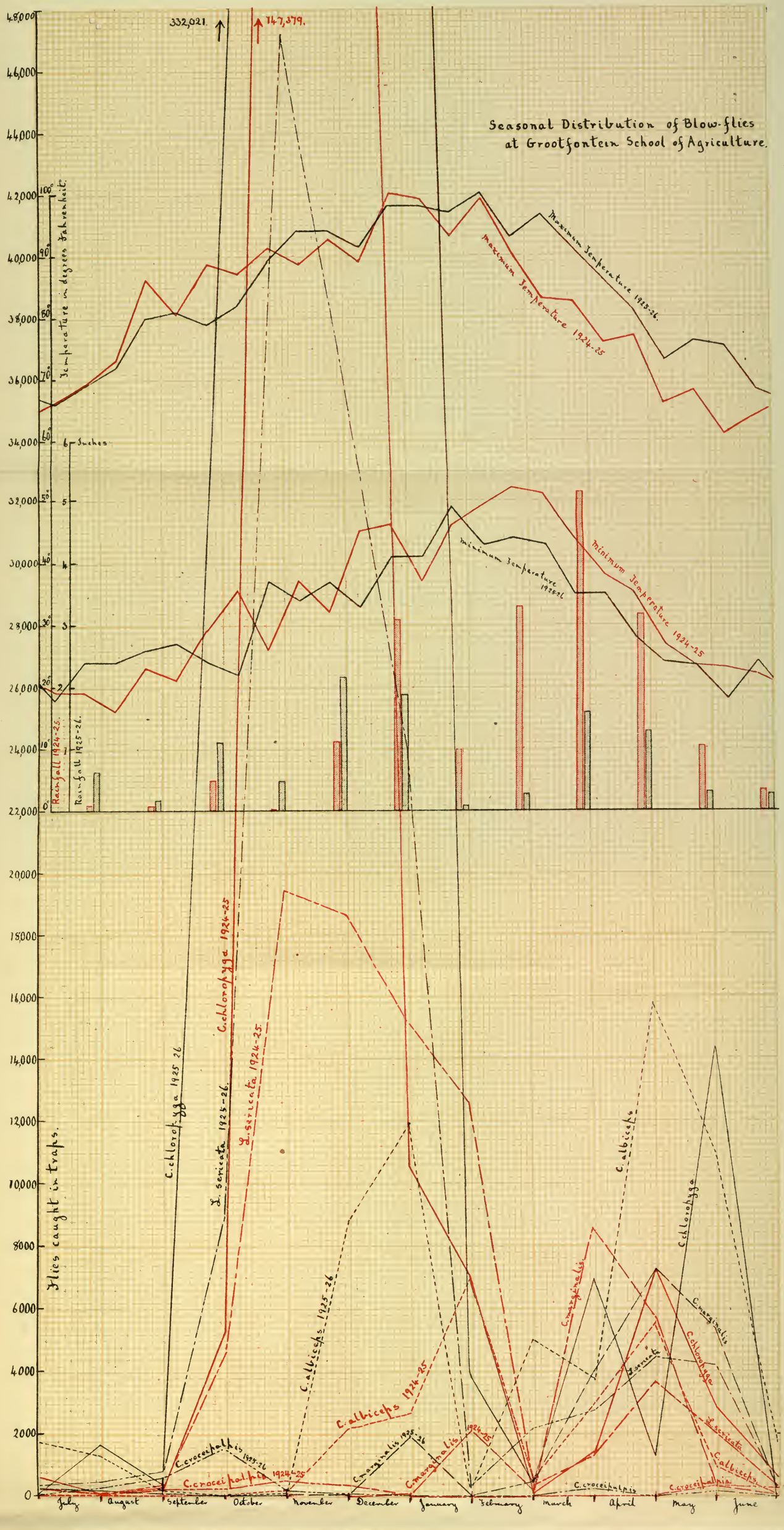
Seasonal Distribution.

It is a most interesting fact that the different species of blow-flies vary enormously in numbers at different seasons of the year and that each species has definite periods when it is most abundant. As shown in the previous chapter some species are far more abundant than others throughout the entire year. Several species of the flies disappear entirely during certain months in the Karroo area. They appear again at almost exactly the same time each year, multiply to their maximum, and then disappear. It is a problem of great interest and importance to discover what happens to these flies during the interval between their disappearance and reappearance and to discover the reasons for their seasonal frequency. Wardle (167) has contributed much valuable information on this subject but as he studied it in Manchester, England, many of his conclusions do not seem to apply here in South Africa.

Referring again to the trapping records in Tables II to VI for the 1924-25 season and to Tables VII to XI for the 1925-26 season, and especially to the totals of all flies caught by the traps during each month of the two years, it will be seen that the periods of abundance of the various species are very different. This is illustrated in Chart No. 1.

DIAGRAM No. I

Seasonal Distribution of Blow-flies at Grootfontein School of Agriculture.



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Small fluctuations in the numbers of the flies are not shown because the traps were only baited and emptied once a month. Wardle believes that in England the periods of maximum abundance of blow-flies correspond to the maxima of emergence of successive generations. The above records were not taken sufficiently frequently to show whether this is so in South Africa or not but our life history studies lead to the conclusion that this is not the case. In England there are only four generations of the blow-fly, *Lucilia sericata*, in the year whereas in South Africa there are nine. During the summer months eggs are laid every day and the overlapping of generations is so complete that the flies emerge in a steady stream.

The condition of the bait has much to do with the numbers of flies caught but great care was taken to bait all the traps uniformly. Each trap contained about ten pounds of bait and this was found to retain its attractiveness throughout the month. The traps were all in the sun.

Undoubtedly weather conditions are the most important factors influencing the abundance of the flies, particularly by affecting their oviposition, emergence and mortality. The trapping records given in the above tables give only a general idea of the seasonal distribution and this will now be discussed.

Referring to Chart No. 1—the curves in red represent the seasonal abundance of the various species of blow-flies during the 1924-25 season and those in black during the 1925-26 season. It will be remembered that more traps were run during the latter period so that the two sets of curves are not comparable as to the relative numbers of flies caught but only in regard to the fluctuations in abundance of the flies.

It is at once apparent that the curves for the two seasons are remarkably similar, the periods of abundance and scarcity being almost the same each year. The almost total disappearance of the flies in the summer, however, occurred at the end of February in 1925 and at the end of January in 1926. A comparison of the meteorological data for the two periods; December, January and February of the 1924-25 summer and the 1925-26 summer throws some light on the reason for this. The temperatures during the two periods do not seem to have affected the numbers of the flies directly. During both periods the maximum temperature curves are very similar except that in the 1924-25 summer the extreme maximum of 100° F. was reached sooner than in 1925-26. It has been stated that the adult flies cannot stand high summer temperatures but if it was this factor alone that affected the flies they should have disappeared earlier in the 1924-25 summer than in 1925-26 and this was not the case.

The wind velocities for the two periods were normal for the time of year but there was more wind towards the latter part of January and in February, 1926, than during the same time of the previous year, the maximum anemometer reading for January being 157 and for February 203 miles per day, whereas the maxima for January and February of 1925 were 139 and 130 respectively. The maximum December readings were both 150. Of far more significance are the rainfall records and consequent evaporation figures. During 1924-25

the rainfall for the three months in question was 7.33 inches whereas during the same period of the 1925-26 summer it was only 2.22 inches.

The evaporation off the surface of standing water for the two periods was as follows:—

		Evaporation in inches.
I.	1924: December	8.07
	1925: January	8.36
	1925: February	5.69
	TOTAL	22.32
II.	1925: December	10.43
	1926: January	10.50
	1926: February	8.95
	TOTAL	29.88

It has been noticed in the insectary that during hot, dry, windy weather the adult flies in cages die off very quickly even though they are constantly supplied with sugar water.

It is, therefore, believed that the combination of hot weather and dry wind such as was experienced in December and January of the 1925-26 summer was responsible for killing off the adult flies sooner than in the previous summer. Dry wind during cool weather does not have the same effect, for the windiest months in the Karroo are August, September and October. These months are very dry but the temperatures are low and the flies multiply very rapidly at this time of the year.

All the flies become very scarce in the winter although some species do not entirely disappear. It is commonly believed that the adult flies are merely killed by the cold. This does not appear to be the case, however, for flies can be kept in cages during very cold weather for months: Life history studies have cast much light on this problem and it is not as simple as it at first appears, there being a number of factors involved.

In the first place, cold weather retards the development of the flies in all their stages and the emergence of the flies from their puparia ceases altogether until about the middle of August, when the weather becomes warm again. They do not lay eggs during the cold winter months.

A large proportion of the eggs that are laid in the late autumn fail to hatch for reasons given in a later chapter and many of the larvae that do hatch die within the first day or two. The development of the larvae is very slow in autumn and many fail to pupate.

The maggots after leaving the meat wander about until they finally shrivel up.

In the case of *C. albiceps* a large proportion of the maggots appear to die just after pupation in autumn.

We see, therefore, that there are two main periods of abundance each year for these blow-flies, the first during the summer and the second in autumn. Some *C. chloropyga* and *L. sericata* were caught during each month of both years and some *C. marginalis* during each month of the second year. *C. chloropyga*, the most abundant and harmful sheep blow-fly, became very numerous during October, November and December and again to a lesser extent during March, April and May.

The numbers of this fly caught in the spring were so great that it was not feasible to construct a graph that would fit in the page and be on the same scale as those for the other species. The height of the curve would have had to be over seven times as high as that for *L. sericata*.

L. sericata came next in order of abundance during the same period in early summer. The maximum autumn abundance of *L. sericata* occurred in April and May but it was then much less numerous than in the spring. It was not as numerous as either of the other sheep blow-flies. *C. albiceps* is sometimes found right through the winter but not in the spring until the end of October. During November, December and January it was abundant, but not to the same extent as the other two sheep blow-flies. Its period of maximum abundance was during April and May when it was the most numerous of all the flies.

C. marginalis is also an autumn species. It does not appear at all in the early summer. During December and January it was found in small numbers and then after disappearing in February became abundant during March, April and May, when it is sometimes the most common blow-fly.

C. croccipalpis is an early spring and late autumn species. It is only seen during comparatively cool weather and reaches its maximum abundance during September and October, occurring again in March, April, May and June.

One of the chief factors influencing the numbers of these flies and particularly the three species of sheep blow-flies is undoubtedly the presence of suitable carrion in which they may breed. Large masses of carrion offer more suitable conditions than small pieces because they do not dry up so quickly and the atmosphere in the crevices is more suitable for the hatching of the eggs. The numbers of eggs laid is also to a large extent proportional to the amount of food available for the maggots. In a large carcase huge masses of eggs are usually laid while in a small animal comparatively few are found.

In the Karroo dwarf flies are seldom seen in Nature. Where too many eggs are laid in a carcase there is great competition between the resulting maggots and when they are about a quarter of an inch long the excess, probably the weaker ones, wander away to shrivel up leaving the others to attain their full size. This is often seen in our breeding jars in the insectary with *C. chloropyga* and *L. sericata*. In the case of *C. albiceps* the maggots are cannibalistic, the stronger ones feeding upon the weaker until the number is reduced to the optimum for the available meat.

Habits of the Flies.

The habits of the adult flies are interesting for many reasons, and a brief discussion of them here will be helpful before we discuss their life-histories.

The flies are very strongly attracted by certain odours—particularly those emanating from decaying animal matter, and therefore chemotrophic responses play a very important part in determining their activities. They are attracted to feed and oviposit.

Feeding.

The natural food of the flies is meat juice, and they may be seen to suck it up eagerly from any carcass left exposed to them. Evil-smelling juices from carrion in an advanced stage of decomposition appear to be as acceptable to them as fresh blood. The flies are fond of any sweet liquid and take sugar water readily. They are often seen swarming round trees infested with aphids from which they lap up the honeydew voided by these insects. A certain amount of moisture in the form of sweet liquid or meat juice is essential to the life of the flies. Without it they die within a few days. If given sugar water they will often drink to repletion and then regurgitate a portion of it so that the liquid may be seen hanging under the proboscis in a large spherical drop. Fly cages become very dirty and sticky on account of the deposition of these drops of sugar water on the netting. This has been observed in the case of the house-fly, and it is said to be one of its most dangerous methods of carrying disease germs—Graham Smith (58). The same author found that a blow-fly could vomit .01 c.c. of syrup at one time.

Maggots feeding in meat are very attractive to blow-flies, and the flies may often be seen feeding on the liquid meat "broth" which the maggots have produced.

As is well known, these flies are strongly phototrophic. They are great lovers of sunlight and become very active under its influence. They very seldom enter buildings and do not go into the shade unless in response to some strong olfactory stimulus or for shelter in bad weather.

In the insectary cages the flies always cluster on the light side and if possible in the sun. They will enter dark crevices amongst carcasses in order to oviposit, however, and in cold windy weather seek shelter at the base of plants.

In confinement they tend to crawl upwards.

Climatic conditions have a great influence on the activities of blow-flies, and particularly is this true of temperature. At 32° F. they are very sluggish and appear sometimes as if dead. The warmer the atmosphere the more active the flies become, emitting the characteristic buzzing sound when flying.

The flies are able to fly long distances and are apparently attracted a long way by carrion. Recent Australian experiments have proved that *C. rufifacies* can fly at least ten miles within twelve days. This question has not yet been investigated in South Africa.

Oviposition Responses.

The chief occupation of the female flies is to lay eggs and most of their activities are connected with this function. Instinct leads the flies to deposit their eggs when and where conditions are most favourable to their development. The attraction to carrion over long distances is undoubtedly olfactory, but when the fly arrives on the bait various other factors come into play. The fly commences by feeding and this feeding is a stimulus to egg production and deposition. The atmosphere in moist crevices in carrion is humid and the damp surfaces in such situations help to induce oviposition. The flies seldom lay their eggs on dry exposed surfaces. The flies do not oviposit on surfaces directly exposed to sunlight because the sun dries the surface before the eggs have time to hatch, making it unsuitable for the larvae to commence feeding. Dry wind also has this effect.

That oviposition is largely induced by odour appears to be proved however by the fact that the females of *L. sericata* will lay their eggs in the flowers of the carrion flower (*Stapelia flavirostris*) which grows on the koppies of the Karroo. It has been stated that the blow-fly will oviposit on objects smeared over with asafetida, but here other factors may have been responsible besides odour.

The flesh-fly, *Sarcophaga haemorrhoidalis*, deposits its larvae on the tops of cages and breeding jars in which blow-fly maggots are developing, and if great care is not taken, these larvae will fall through into the meat and develop at the expense of the rightful occupants. Calico is now used over these jars and although the flesh-flies will deposit their larvae on this, the small maggots cannot get through it. Fabre (38) found that the blow-fly *Calliphora vomitoria*, L., would lay its eggs on a variety of substances such as coloured paper, oil-skin and tin-foil when placed over a receptacle which contained meat but only when there was an opening through which the maggots, which hatched from the eggs, could get to the meat and develop. Our own experiments confirm this.

A wide mouth fruit jar was half filled with meat and over its top was tied a piece of wire gauze, 14 mesh to the inch, so that no flies could get into the jar. The jar was then exposed outside the insectary. A few days afterwards two masses of eggs were found stuck to the glass just inside the mouth of the jar. They had been laid through the gauze. Another mass was found on the gauze in the centre of the cover. There were also many eggs on the meat itself scattered singly over its surface. All these eggs hatched and the maggots developed in the meat. They were *L. sericata* maggots.

When meat is dried up it no longer attracts the flies. Its odour diminishes and changes in nature, and other insects such as the Dermestid beetles take up the work of reduction.

The oviposition responses to living sheep are of great practical interest, but the problem here is more difficult to study and there are many obscure factors involved. Although, as has been shown, the period of maximum infestation of live sheep corresponds in most places with the period of maximum abundance of the flies, the abundance of the flies is not always the chief factor in inducing infestation.

Five Merino sheep were kept in a large gauze cage for a week with over 500 *L. sericata* flies. These flies were mature and had been layings eggs on meat in one of our cages for three weeks. One of the sheep was in a sickly condition and inclined to scour. Its wool was wet and soiled under the tail and it appeared to be in ideal condition to be infested. On the third day a piece of meat was exposed on a shelf in the cage and the flies laid large masses of eggs on it but the sheep remained uninfested. On the fourth day a large number of *C. albiceps* taken from a fly cage were liberated in the cage with the *L. sericata*. On the fifth day the sheep were swabbed behind with water until the wool was soaked, and they remained wet until the following day. They were again swabbed on the sixth day. On the seventh day a number of the *C. albiceps* flies laid eggs on meat in the cage as well as *L. sericata*, but the sheep were not struck. This was in the middle of February, and the weather was very dry. The sheep were then taken out for use in another experiment, but later in the year when this experiment was repeated, several of them became infested.

It appears that weather conditions are the most important factors that influence the attack of the flies on the sheep. The weather must be warm and damp, but sheep are often struck in the Karroo as late as the end of June on warm days although the nights are frosty at that time of the year. A certain amount of moisture in the atmosphere seems essential and it is common knowledge amongst sheep farmers that the attack of the flies is worse during rainy seasons than during droughts. It is also worse in areas where rain is plentiful than in the dryer parts of the country. A combination of warmth and moisture is therefore necessary but moisture is probably the more important of the two.

On the other hand, during January and February of 1930 copious rains fell in the Karroo and weather conditions seemed ideal for the attack of the flies. There was plenty of green grass and a flock of experimental sheep with wool three inches long about the crutch got into a filthy state. It was expected that they would become infested but they remained healthy until the end of March. This was probably due to the absence of the flies which had died out during short spells of very hot weather in January.

Only Merino sheep are attacked, and in the great majority of cases the flies lay their eggs on the wool in the " crutch " which is that part between the hind legs and below the tail where the wool is liable to become soiled with dung and urine. They will also attack wounds such as shear-cuts or the blood-stained wool at the base of the horns of rams after they have been fighting. The eyes of sheep are often blown after they have been injured by grass seeds such as " steekgras " and are in a watery condition. Ewes are often struck just after lambing on account of the discharge from the vagina at that time and lambs need special treatment after their tails have been docked to prevent the stumps becoming infested. In short, any damp evil smelling discharge or excrement on the sheep tends to attract the flies and induce oviposition.

One of the effects of damp weather is to keep the soiled wool moist and in an attractive condition. After it has dried the flies will not oviposit on it.

Some sheep seem to be particularly attractive to the flies and are repeatedly attacked. They are usually very fine woolled sheep carrying a dense fleece, but in addition to this there appear to be other characteristics which add to their attractiveness.

Secretions from the skin causing the wool to be damp and an unhealthy condition of the bowels causing a discharge or scouring predispose to the attack of flies. In South Africa scouring is often caused by infestation of intestinal round worms. Luscious green fodder tends to soften the excrement and so soil the wool in the crutch. This is one reason why sheep running on lucerne lands or feeding on green barley or oats are often badly infested with maggots. Cactus has an even more marked effect in this respect and sheep fed on it need to be watched carefully. Green oats also appears to affect the urine, causing it to burn the skin so that it becomes inflamed along the folds beside the vulva. In some cases the skin becomes raw and bleeds before infestation occurs.

The flies will strike healthy sheep on any part of the body at certain times. This is usually during wet periods when the wool is kept damp for several days, or when sheep are run in damp situations. Wrinkly sheep are more susceptible to attack than smooth sheep simply because the wool between the folds of the skin retains the natural secretions and does not dry quickly. For the same reason rams with their horns coiled tightly against the head get maggots in the wool under the horns.

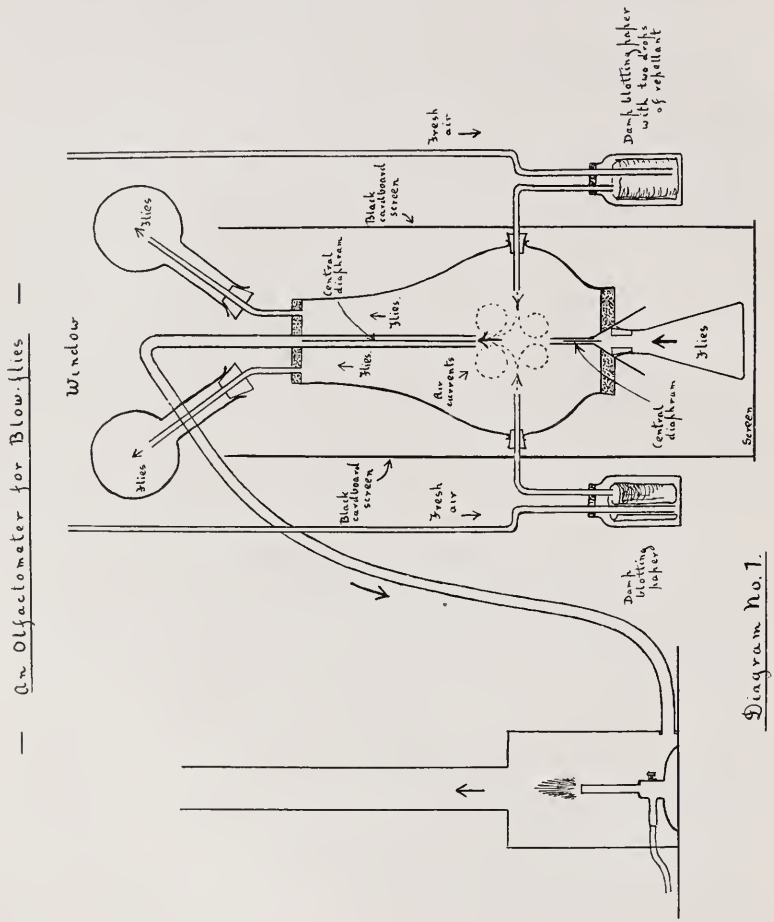
In South Africa, natives in shearing and crutching often clip off the tips of the labiae of the vulva in ewes so that afterwards the urine is not carried clear of the wool but tends to run down the inside of the legs. The wool in the crutch of such sheep becomes almost rotten by being constantly damp. The skin also becomes red and inflamed and the sheep is very likely to be struck.

The length of the wool is an important factor although only indirectly. Sheep that have just been shorn are not often attacked except in shear wounds, because the short wool does not easily get soiled and dries quickly. After the wool is about an inch long it may become attractive and it becomes more so as it grows longer. Its attractiveness does not increase in proportion to its length. A number of sheep were left for two years without shearing and at the end of that time the wool was nearly five inches long, but they were not more severely attacked the second year than the first. As stated above, blowfly maggots and the secretions they produce are particularly attractive to the flies and an infested sheep is very subject to repeated attacks. The attraction remains long after the maggots have been removed and it is for this reason that a dressing used on infested sheep should have a lasting repellent action.

Fly Repellents.

It appears to be a very difficult matter to obtain definite data on the attractant and reppellent action of various substances on blow-flies. Repeated attempts have been made but so many factors are involved that the results have been unreliable and sometimes contradictory.

Our first experiments were made with an olfactometer set up in the laboratory at an open window. The principle of the apparatus was the same as that used successfully by McIndoo (96) for testing the reactions of potato beetles to odours but constructed on a much larger scale out of a large lamp chimney as shown in diagram No. 1.



A bunsen burner was used to create a draught through the apparatus and the flies were attracted through it by light from the window. The results were disappointing and after many trials and adjustments all that could be shown was a positive chemotrophism to meat. The flies were too eager to escape towards the light to be influenced to any great extent by the odours. There appeared also to be too much mixing of the odours across the central diaphram.

Ripley of Cedara has devised a more successful apparatus and the author hopes to try a similar apparatus for blow-flies at some future date. Ripley and Hepburn (136).

Meat jars exposed to the flies out of doors were next tried. Repellent mixtures were poured over the meat in the jars but here again there were so many factors involved that the results were unreliable, although some interesting observations were made. The method was similar to that of Parman, Bishoff, Laake, Cook and Roark (119). Battery jars of two quarts capacity were used. A piece of beef weighing one and a half pounds was put into each jar and 40 c.c. of the oily repellent was poured over it. Nine mixtures were tested simultaneously using two jars A and B to each mixture and two check jars. These were set out in a large cricle thirty yards in diameter, the jars being about five yards apart, and the two jars containing the same repellent opposite each other. The positions of these jars were interchanged across the circle each morning to eliminate the effect of the wind. The experiment was started at the beginning of January when the weather was hot. Good rains had fallen and the flies were abundant. Observations were made twice a day, at 9 a.m. and 4 p.m., and the number and species of flies in or on the edge of each jar recorded. The development of any maggots in the jars was also recorded. The results are given in Table XIII.

It is believed that the very marked repellent action was more due to mechanical factors than to the odours of the repellents. The meat lay in the oily liquids and was more or less covered with oil so that its surface was in an unnatural state. The oil also prevented the meat from drying and kept it in a very wet, stinking condition. Its decomposition was affected, probably by a change in its bacterial flora. In some of the jars the meat was covered by an oily liquid consisting of meat juice and oil at the end of the experiment.

The check jars were freely visited and the meat soon consumed by maggots. The same happened in the case of the meat treated with glycerine. Fish oil did not altogether prevent flies visiting or maggots developing in the meat. The half and half mixture of cotton seed oil and paraffin did not repel very well, but all the other cotton seed oil mixtures kept the flies away. Carbolic acid, oil picis and pyridine were the best repellents.

The next series was run with the jars supported on short poles about three feet from the ground to avoid disturbance from rats, etc., and the repellent mixtures were not poured over the meat but put in small tins which were hung in the mouths of the jars above the meat. One and a half pounds of meat was used in each jar as before and 30 c.c. of repellant in each tin. The arrangement of the jars in a circle and the interchange of positions each morning was the same as in the first series, also the method of recording results. The mixtures were the same except for the substitution of Stockholm tar for glycerine. The results are shown in Table XIV. The check jars were freely visited. Fish oil did not repel to any great extent. The

best repellent was pyridine. Only four flies were seen in the pyridine jars and no eggs were laid in them. Carbolic acid seemed almost as good but oil picis failed to keep the flies from ovipositing in the jars. The other mixtures also failed in this respect.

In this case the mechanical factors referred to in connection with the first series of tests were not present, only the odours of the mixtures producing the above results. The jars were examined twice daily and the total number of flies of each species seen in the two visits recorded for the day. This method was unsatisfactory for only those flies that happened to be in the jars at the time of inspection were recorded.

A third series of experiments was therefore carried out using gauze fly traps over the jars to catch the flies that were attracted. In this case only one pound of meat was put into each jar. The small tins containing 30 c.c. of the repellent mixtures were used as before and over the top of the jar was fitted a bell fly trap. The whole was then supported on a stand as shown in the accompanying photograph. Twenty jars were used as before, two checks and two for each repellant and they were set out in a circle as above described. This was at the end of April. Good rains had fallen and the weather was ideal. The flies were also very abundant. The positions of the traps were interchanged across the circle each day. After fourteen days the meat had ceased to smell and had dried up. The weather turned cold and flies became scarce. The traps were therefore taken up and the flies in each counted. The results however were confusing. They are given in Table XV.

These results are quite unreliable and are given only to show the difficulty of such work. Every precaution was taken to see that all the traps were identical in size, shape and setting, but although their positions were interchanged daily some of the "A" traps caught over ten times as many flies as the "B" traps and *vice versa*. Apparently certain other factors were present which had not been controlled. Probably the decomposition of the meat in the various jars was not of a uniform nature and the attractiveness of the traps varied on this account. A second series of these experiments was run in November, using the traps again, but no better results were obtained. Evidently some standard attractent is required instead of the decaying meat.

A fly-trap on stand as used in the repellent experiments. A small tin containing the repellent was set just inside the bait-tin seen below the trap.

TABLE XIII.

Jars.	Observations.													Total.	Remarks.
	January.										February.				
	10.	11.	12.	13.	14.	15.	16.	19.	23.	28.	6.	13.	18.		
No. 1. A. and B. Cotton seed oil 90 % Carbolic acid 10 % Sheep blow-flies.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No maggots developed in meat. On January 9th hundreds of Lygaeid bugs were caught in both jars.
No. 2. A. and B.— Cotton-seed oil 50% / Fish oil 50% Sheep blow-flies.....	0	0	0	*One	magg	ot in	meat.	Meat	very o	ily.	0	0	0	1	The maggot did not mature. Meat in A remained oily. Meat in B dried up.
No. 3. A. and B.— Pure Fish oil Sheep blow-flies.....	0	0	0	*A 5	few m 0	aggots 0	in mea 0	t. 0	0	0	1	0	0	6	A few maggots matured. Meat in A. remained very oily. Meat in B. dried up.
No. 4. A. and B.— Cotton-seed oil 90% Oil Picis 10% Sheep blow-flies.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No eggs laid on meat. Meat in A. remained very oily. meat in B. dried up.
No. 5. A. and B.— Cotton-seed oil 90% Fusil oil 10% Sheep blow-flies.....	0	0	0	0	0	0	0	1	0	0	0	0	0	1	No eggs laid on meat. Meat in both jars remained almost covered with oily liquid.

TABLE XIII.—*Continued.*

Jars.	Observations.													Total.	Remarks.
	January.										February.				
	10.	11.	12.	13.	14.	15.	16.	19.	23.	28.	6.	13.	18.		
No. 6. A. and B.— Cotton-seed oil 90% Pyridine 10% Sheep blow-flies.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No eggs laid on meat. Meat in both jars remained covered with oily liquid.
No. 7. A. and B.— Cotton-seed oil 50% Paraffin 50% Sheep blow-flies.....	0	0	0	1	2	0	0	0	6	0	1	0	0	10	No eggs laid on meat. Meat oily, but drying up at end of experiment.
No. 8. A. and B.— Pure glycerine Sheep blow-flies.....	3	5	3	11	1	*A few maggots in meat. 0	0	0	0	0	0	0	0	23	Many maggots matured and consumed the meat in both jars.
No. 9. A. and B.— Pure cotton-seed oil Sheep blow-flies.....	0	0	0	1	0	1	0	0	0	0	1	0	0	3	No eggs laid on meat. Meat in both jars remained almost covered with only liquid.
No. 10. A. and B. Checks Meat left untreated.... Sheep blow-flies.....	4	*Many maggots in both jars. 16	4	41	0	4	2	Meat consumed.						71	Many maggots matured and consumed all the meat in both jars.

TABLE XIV.

Jars.	Observations. February. 19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	Total.	Remarks.
No. 1.—												
Cotton-seed oil 90%												
Carbolic acid 10%												
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		Meat dried up by the 27th and stopped smelling. Could still smell the carbolic above the jars. No maggots developed in either jar.
L. sericata.....	—	—	1	—	—	—	—	—	—	—		
C. albiceps.....	—	1	—	—	—	—	—	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	1	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	—	2	—	—	—	—	—	—	—		
Totals.....	1	1	3	0	0	0	0	0	0	0	5	
No. 2.												
Cotton-seed oil 50%		*Large mass of eggs on meat.										
Fish oil 50%												
A.—*C. chloropyga*.....	—	1	—	—	—	—	—	—	—	—		Eggs laid in jar B., but did not develop. Meat dried up at end of experiment.
L. sericata.....	—	1	1	—	—	—	1	1	—	—		
C. albiceps.....	—	2	2	—	—	—	—	2	—	2		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	2	—	—	—	—	—	—	—	—		
C. albiceps.....	—	2	4	—	1	—	—	—	—	—		
Totals.....	0	8	7	0	1	0	1	3	0	2	22	
No. 3.—												
Pure fish oil	*Mass of eggs on meat.											
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		Eggs in both jars hatched, but maggots died when half-an-inch long. Meat dried up.
L. sericata.....	—	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	—	2	—	1	—	—	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	1	—	—	—	—	—	—	—	—		
C. albiceps.....	—	1	1	3	—	—	—	—	—	—		
Totals.....	0	2	3	3	1	0	0	0	0	0	9	

TABLE XIV.—*Continued.*

Jars.	Observations. February. 19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	Total.	Remarks.
No. 4.—												
Cotton-seed oil 90%	*Mass of eggs in both jars.											
Oil Picis 10%												
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		A few maggots developed in and escaped from both jars.
L. sericata.....	—	—	1	—	—	—	—	—	—	—		
C. albiceps.....	—	—	—	—	4	—	1	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	—	—	—	1	—	—	—	—	—		
C. albiceps.....	—	—	—	—	—	—	1	—	—	—		
Totals.....	0	0	1	0	5	0	2	0	0	0	8	
No. 5.—												
Cotton-seed oil 90%	*Mass of maggots in Jar A.											
Fusil oil 10%												
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		A few maggots developed in and escaped from Jar A. Eggs were laid in Jar B., but the resulting maggots died when ½-inch long.
L. sericata.....	—	—	1	—	—	—	—	—	—	—		
C. albiceps.....	—	1	3	1	—	—	1	1	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	1	—	—	—	—	—	—	—	—		
C. albiceps.....	—	1	—	—	—	—	—	—	—	—		
Totals.....	0	3	4	1	0	0	1	1	0	0	10	
No. 6.—												
Cotton-seed oil 90%												
Pyridine 10%												
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		No eggs were laid in meat in either jar.
L. sericata.....	—	—	—	—	—	1	—	—	—	—		
C. albiceps.....	1	—	1	—	—	—	—	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	—	—	—	—	1	—	—	—	—		
C. albiceps.....	—	—	—	—	—	—	—	—	—	—		
Totals.....	1	0	1	0	0	2	0	0	0	0	4	

TABLE XIV.—*Continued.*

Jars.	Observations. February. 19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	Total.	Remarks.
No. 7.—												
Cotton-seed oil 50% Paraffin 50%			*Mass of eggs in Jar B.									
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		A few maggots developed in Jar B. and escaped.
L. sericata.....	—	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	—	—	—	—	—	3	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	—	1	—	—	—	—	1	—	—		
Totals.....	0	0	1	0	0	0	3	1	0	0	5	
No. 8.—												
Pure Stockholm tar			*Mass of eggs in Jar B.									
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		A few maggots developed in both jars until they were ¼-inch long and then died.
L. sericata.....	—	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	—	—	—	—	—	—	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	—	—	1	—	—	—	—	—	—		
C. albiceps.....	—	3	7	—	—	—	—	—	—	1		
Totals.....	0	3	7	1	0	0	0	0	0	1	12	
No. 9.—												
Pure Cotton-seed oil			*Mass of eggs in Jar B.									
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		A few maggots developed in Jar B. and escaped from it. None in Jar A.
L. sericata.....	—	—	—	—	—	—	—	—	—	—		
C. albiceps.....	—	3	5	1	—	—	—	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	—	—	1	—	—	—	—	—	—	—		
C. albiceps.....	—	1	1	1	—	—	—	—	—	—		
Totals.....	0	4	7	2	0	0	0	0	0	0	13	

TABLE XIV.—*Continued.*

Jars.	Observations. February. 19	20	21	22	23	24	25	26	27	28	Total.	Remarks.
No. 10.— Checks. No repellent put in jars			*Masses of maggots in Jar A. and B.									
A.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		Maggots developed in and escaped from both jars.
L. sericata.....	1	1	1	—	—	1	—	—	—	—		
C. albiceps.....	—	—	—	2	1	—	1	—	—	—		
B.—*C. chloropyga*.....	—	—	—	—	—	—	—	—	—	—		
L. sericata.....	1	—	2	—	—	—	—	—	—	—		
C. albiceps.....	—	1	7	6	3	—	1	3	—	5		
TOTALS.....	2	2	10	8	4	1	2	3	0	5	37	

TABLE XV.

Traps.	Flies Caught in "A" Trap.				Flies Caught in "B" Trap.				
	C. chloropyga.	*L. sericata.*	*C. Albiceps.*	Totals for each Trap.	*C. chloropyga.*	*L. sericata.*	*C. albiceps.*	Totals for each Trap.	Totals for both Traps.
No. 1. Cotton-seed oil 90 % Carbolic acid 10 %	102	169	422	693	12	13	66	91	784
No. 2. Cotton-seed oil 50 % Fish oil 50 %	16	26	86	128	110	104	437	651	779
No. 3. Pure fish oil	39	24	110	173	15	52	75	142	315
No. 4. Cotton-seed oil 90 % Oil picis 10 %	9	8	35	52	123	100	460	683	735
No. 5. Cotton-seed oil 90 % Fusil oil 10 %	14	11	83	108	14	24	70	108	216
No. 6. Cotton-seed oil 90 % Pyridine 10 %	51	110	284	445	4	7	20	31	476
No. 7. Cotton-seed oil 50 % Paraffin 50 %	12	0	41	53	22	8	82	112	165
No. 8. Pure Stockholm tar	40	46	83	169	36	34	74	144	313
No. 9. Pure cotton-seed oil	15	20	30	65	129	163	663	955	1,020
No. 10. Checks. No repellent in traps	97	147	337	581	6	62	24	92	673

Protection of Sheep.

One hundred young Merino ewes were selected from a flock of over five hundred as being those with the most pleats or folds over the body. Many had, what sheep men call, "Rose" tails and the folds between the hind legs were in most cases well developed. They were thus the sheep most susceptible to the attack of the flies.

The sheep were divided into five equal groups marked A, B, C, D and E, containing twenty each and they were numbered from one to a hundred consecutively. Group A was left untreated and served as the check lot. The sheep in Group B were crutched; the wool over the tail and between the hind legs being shorn away so that the whole region around the anus and vulva was bare.

Group C was jetted with a commercial sheep dip which is often recommended for the purpose. The yellow powder was mixed at the rate of one packet (2 pounds) to seven gallons of water.

Jetting consists of squirting a solid stream of the dip between the hind legs of the sheep. The method has been largely used in Australia where two thousand sheep are sometimes jetted in a day. A standard jetting pump such as is being sold for the purpose in South Africa was used. It was fitted with a pressure gauge and the average pressure during the operation was found to be sixty-five pounds per square inch. The nozzle had an opening $\frac{1}{8}$-inch in diameter so that a fairly heavy stream was delivered. The jetting was done more thoroughly than is usual in Australia, each sheep being turned over on its back and the area between the hind legs well soaked with the dip. It was then allowed to stand up and the area around and above the tail saturated. The sheep had four months' growth of wool on them and the penetration of the dip was very satisfactory.

Group D was jetted with a solution of pure arsenite of soda, 14 oz. in ten gallons of water which is the strength recommended in Australia. This solution penetrated the wool a little better than the dip. The jetting was done very thoroughly as described above, ten gallons of the solution being used to do twenty sheep.

The sheep in Group E were dressed around the tail and between the hind legs with the following mixture:—

Raw linseed oil	75	parts	by	volume
Pine oil (oil picis)	10	,,	,,	,,
Petrol	10	,,	,,	,,
Carbolic acid	5	,,	,,	,,

Each sheep was laid on its back and the mixture poured into the wool and worked well down to the skin with the fingers. One gallon of the mixture was used to do the twenty sheep.

All the sheep were then turned out to graze on a land where oats were coming up. This was on 27th September and on 30th September heavy rain fell and the sheep soon had plenty of succulent, green feed. L. sericata became very abundant and began to attack the sheep on 2nd October. The attack of the flies was particularly severe, eighty of the sheep becoming infested before the end of the month. The sheep were carefully examined each day and those found with maggots recorded. The results are given in Table XVI.

Each sheep was kept under observation and its condition noted daily. In some cases only a few maggots developed in the wool and it recovered without treatment. In most cases the sheep became badly infested and it was necessary to dress it in order to save its life. It was then counted out of the experiment and used for the testing of dressings.

After 2nd December no more of the sheep were attacked. This was because the weather became very dry and the flies disappeared. Eighteen of the twenty control sheep became infested and only one of these recovered without treatment. They were attacked early in the experiment. Those sheep that were jetted were just as heavily infested as the controls. They were, in fact, infested sooner than the controls, but a much larger proportion of them recovered without dressing.

The pure arsenite of soda proved slightly more effective in this respect than the dip. The sheep that were dressed with the mixture remained healthy much longer than the controls or those that were jetted although in the end seventeen became infested and sixteen of these had to be treated. The dressing had a very marked repellent effect however. The crutching proved to be by far the best method of protecting the sheep. Only twelve of the sheep were attacked and six of these towards the end of the experiment when the wool about the tail had grown again.

The conclusions are that crutching is the most effective method of protecting the sheep and the dressing has a marked repellent effect but that jetting does not prevent the sheep from becoming infested. The arsenical in the wool, however, tends to destroy the maggots and so helps the sheep to recover from myiasis without further treatment.

Note.—The above mixture does not kill maggots in the wool of a sheep as quickly as is necessary and the proportion of petrol has therefore been increased to 40 per cent. in the dressing recommended to farmers. The proportion of linseed oil or cotton seed oil is reduced to 45 per cent. so that the proportion of the other two ingredients remain unchanged. This makes a very excellent and inexpensive dressing for infested sheep.

TABLE XVI.

Dates	October. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Totals for October.	November. 2	4	8	18	Dec. 2	Totals for the Experiment.
Group A. Number of sheep infested.....	–	1	–	–	–	–	–	4	–	6	4	1	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	18	–	–	–	–	–	18
Number so badly infested that they had to be dressed	–	–	–	–	–	–	–	1	1	–	1	5	–	6	–	2	–	–	–	1	–	–	–	–	–	–	–	–	–	–	17	–	–	–	–	–	17
Number that recovered without dressing.....																															1						1
Group B. Number of sheep infested.....	–	1	–	–	–	–	–	1	–	2	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–	2	–	1	–	– 9	–	–	1	–	2	12
Number so badly infested that they had to be dressed	–	–	–	–	–	–	–	1	–	–	1	1	–	1	–	1	–	–	–	–	–	–	–	–	–	–	1	–	2	–	– 8	–	–	1	–	1	10
Number that recovered without dressing.....																															1						2
Group C. Number of sheep infested.....	–	1	–	–	1	–	–	8	4	2	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	– 18	–	–	–	–	–	18
Number so badly infested that they had to be dressed	–	–	–	–	–	–	–	2	1	4	2	–	–	1	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	1	13	–	–	–	1	–	14
Number that recovered without dressing.....																															5						4
Group D. Number of sheep infested.....	–	–	–	–	1	–	–	9	4	3	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	18	–	–	–	–	–	18
Number so badly infested that they had to be dressed	–	–	–	–	–	–	–	1	1	2	1	3	1	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	11	–	1	–	–	–	12
Number that recovered without dressing.....																															7						6
Group E. Number of sheep infested.....	–	–	–	–	–	–	–	–	2	1	1	–	4	–	3	1	–	3	–	1	–	–	–	–	–	–	–	–	1	–	– 17	–	–	–	–	–	17
Number so badly infested that they had to be dressed	–	–	–	–	–	–	–	–	–	–	–	1	1	1	3	–	–	6	–	1	–	–	–	–	1	–	–	–	1	–	– 15	1	–	–	–	–	16
Number that recovered without dressing.....																															2						1

PART II.

LIFE-HISTORY STUDIES.

Conditions Affecting the Development of Blow-fly Eggs.

The eggs of the sheep blow-flies are very susceptible to climatic conditions and will not develop unless these conditions are favourable. Considerable difficulty is sometimes experienced in getting them to hatch under artificial conditions. The chief factors influencing their development are temperature and moisture. Their development is hastened by warmth as shown in the accompanying life-history diagrams and retarded by cold, but the extremes of temperature experienced in the high Karroo area are not directly injurious to them. They will hatch when the thermometer registers 100° F. in the shade and also after freezing night temperatures. Excessively dry air is directly injurious to them and causes them to shrivel and die. If a batch of eggs is left exposed on a table indoors in the spring when the weather is dry and dusty the eggs do not hatch but shrivel up. They will even do so if left exposed on the dried surface of a piece of meat and the author has sometimes attempted to infest the wool of live sheep artificially during the dry periods of the year by tying batches of fresh eggs in their wool but without success. The eggs shrivel and do not hatch. The same applies to eggs laid on dry cotton wool. On the other hand if eggs are placed near a plug of wet cotton wool and covered with a bell-jar they hatch in a clean petri dish and the resulting maggots will crawl over the table for a distance of five or six inches. If uncovered after hatching the maggots live for four or five hours without food or moisture. On a hot, dry day in the Karroo they live for about three hours on a bare table. If the egg mass is large, merely covering it with a petri dish is enough to induce many of the eggs to hatch on account of the moisture contained in the mass. In ovipositing, the fly chooses a locality where the atmosphere contains the optimum amount of moisture. This is found in cavities in meat or round the lower edge of a piece of meat lying in a dish where the surface remains moist and yet not wet and where the maggots on hatching can immediately reach soft, wet tissue on which to feed. On the other hand, excessive moisture is very detrimental to blow-fly eggs. If for any reason they become immersed in meat juice they fail to develop, losing their shiny, waxy appearance, turning a dirty yellow colour, and finally rotting. Apparently they are drowned. Healthy eggs are not easily wetted because of their waxy surface which causes water to remain in round drops and roll off them. Presumably, this is a provision of nature. If a piece of juicy meat is placed on an egg mass and immediately removed so that the eggs are allowed to dry at once they usually hatch but if the meat is left on them they rot. The waxy surface of the eggs also protects them from the action of poisons. Eggs on meat were sprayed lightly with a 1 per cent. solution of arsenite of soda. They were not wetted by this treatment and hatched normally. Most of the maggots developed in the meat.

A combination of cool, damp weather, as usually experienced at Grootfontein in the autumn causes a large proportion of eggs laid on carcasses in the open to go mouldy. If put on meat under bell-jars or in airtight glass boxes practically all of them fail to hatch. This is because the hatching of the eggs is delayed by the low temperature and on account of the damp atmosphere they become overgrown with mould. On examining such eggs taken from a carcass in May they were seen under the binocular to contain fully formed larvae but were of an unhealthy cream colour without the characteristic lustre and the mycelium of the mould could be seen all over them. They did not hatch. This may partly account for the disappearance of the sheep blow-flies in the Karroo in the late autumn and winter.

In the insectary it is very difficult to keep the air around the eggs at the correct humidity in autumn. If, on the other hand, they are put into an incubator at 85° F. they hatch very readily before the mould has time to attack them.

Life Histories.

The life histories of the three sheep blow-flies of South Africa are very similar. The flies all lay eggs from which maggots hatch, and these feed ravenously in meat or in the wool of the sheep until they are full grown. They leave the carrion or the sheep to burrow into the soil, where they pupate, and from these puparia the flies emerge.

The two species *L. sericata* and *C. chloropyga* have been reared in the insectary at Grootfontein from one generation to another through a whole year, and their development has been studied in great detail. *L. sericata* is comparatively easy to rear in captivity. *C. chloropyga* is more difficult and *C. albiceps* is so difficult that the author has not yet succeeded in keeping it alive through more than three generations. As shown in a previous chapter, it is the scarcest of the three flies in nature and does not appear at all during several months of the year. Numerous attempts have been made to carry the fly through the period when it disappears in the field, but each time it has died in our cages in the pupal stage or the adults have died without laying eggs. Apparently we have not been able to provide exactly the required conditions.

Technique.

The flies have been reared in an insectary under conditions as natural as possible. The insectary has an iron roof, but under this is a thick layer of reed thatch to keep the place moderately cool. The sides are of wire gauze and on one side of the insectary is a thin windbreak of reeds to keep high winds from sweeping through it too violently.

For rearing *L. sericata*, the Graham-Smith cage was used as described by the author in Science Bulletin No. 68, 1928. This cage was found to be rather cumbersome when several different species of flies were being reared and many cages were needed at the same

time. A new type was therefore devised. The new cages were built in series of six and consisted of a rectangular wooden framework covered with cloth mosquito-netting on top and sides and with a galvanized iron bottom, in which were sheet-metal slide-doors. Food and water were put in for the flies from below as in the case of the Graham-Smith cage. Each cage measured 18 by 18 by 14 inches, and the cages were fastened against the sides of the insectary about 5 feet from the floor. The morning sun was able to shine into them until about 10 o'clock in winter, and the flies would always cluster in the sunlight whenever possible. On very cold nights the tops of the cages were covered with sacks to give the flies some shelter, and they would cluster under these at the top of the cages.

The interior of the author's insectary, showing the fly-cages, bell-jars, and battery-jars in which the flies were reared throughout the year.

It was found necessary to keep the flies constantly supplied with moisture, otherwise they would die of thirst in a day or two, particularly during the hot, dry days of summer. For this purpose 30 per cent. sugar solution was used in petri dishes containing small pieces of cotton-wool to prevent the flies from getting drowned in the syrup.

The sugar-syrup was renewed in the cages daily.

To give additional moisture and food and also to induce the flies to lay their eggs, the cages were kept supplied with fresh meat, lumps of which, weighing about half a pound, were placed in evaporating dishes and renewed each day. The cages were attended to at approximately the same time every morning, and not looked at again during the day unless needing some special attention.

Every morning the meat in the cages was examined for eggs, which were laid in batches, usually in some crevice or in hollows under the meat between it and the dish. The first three batches of eggs found in a cage were taken out in the evaporating dish and put to hatch under a bell-jar. The bell-jars were of a special type made for insect work, with a large opening at the top for ventilation, over which fine muslin or calico was tied. They were of five-gallon capacity, giving sufficient space under them to ensure a plentiful air-supply. Smaller jars were tried at first, but did not answer, as the stench and humidity became so great in them that the maggots were asphyxiated. The bottoms of the jars were ground and stood on a large piece of plate-glass.

It was found necessary to take every precaution that the cultures of maggots did not become mixed, for although the Insectary was fly proof, other species of blow-flies would often enter when the door was opened and if given an opportunity would, lay their eggs on the meat in the cages or bell jars.

This was particularly true of the Grey Flesh-fly, *Sarcophaga haemorrhoidalis*, which is viviparous, depositing its maggots anywhere near decaying meat to which they will crawl through gauze or netting. It has even been found to drop its maggots on to meat in bell jars through their open tops when not properly protected with muslin. The larvae of this fly are so ravenous and strong that they often kill those of *C. chloropyga* and *L. sericata* that are being reared in the meat, if allowed to enter.

Another danger which was avoided by the use of bell jars with ground bottoms on sheets of plate-glass was the attack of the minute Chalcid parasite. *Mormoniella vitripennis*, Walker (*Nasonia brevicornis*, Ashmead), on the puparia of the flies. It was impossible to keep this tiny parasite out of the Insectary as it flies through gauze with ease and is very abundant throughout the summer but it could not get into the bell-jars or the battery-jars described below, when covered with muslin.

When maggots have finished feeding in meat and are full grown they crawl away to find a suitable place in which to pupate. This was provided by placing the meat dish in a larger evaporating dish of 9 inches diameter half filled with clean sand. The sand was previously sifted through wire gauze to prevent the possibility of any foreign fly puparium being introduced.

On leaving the meat dish the maggots fell on to the dry sand, where they cleaned and dried themselves. After this cleaning process they were unable to crawl out of the sand-dish on to the plate-glass. They remained on the surface of the sand for a time and then burrowed under the meat-dish in the centre until they were completely buried. Care was taken that there were not too many maggots in the meat otherwise they would leave it prematurely and fail to attain their normal size.

When all the maggots had left the meat they were removed from the bell-jars and thrown into small battery-jars with the sand from the sand-dishes. This was done to economize in the use of the large bell-jars of which only a few were available. The battery-jars were

of two quarts capacity. After the maggots and sand were poured in, a little extra sand was sifted into the jars to ensure that all the maggots were buried, and small stones were put on to the sand to help them to burrow into it again in case they became uncovered. The battery-jars were then covered with muslin and watched until the flies emerged.

Observations on the time taken by the maggots to pupate were made by removing the covers and stones from these jars each day and pouring the sand away until a few of the maggots could be seen. Their condition was then recorded and the sand, stones, and covers replaced.

The first flies that emerged in any one of the three battery-jars containing maggots from the first three lots of eggs laid in a cage were immediately put into another cage. This was done by standing the battery-jar containing the flies in the cage and removing its muslin cover. The flies were then given sugar solution and fresh meat. The cages were numbered consecutively corresponding to the successive generations of flies which they contained.

If, on the following day sufficient flies had emerged into the cage, from 50 to 100 flies being considered sufficient, the jar was removed. If not, it was left two or three days longer and then removed. The jar was covered with muslin again and records were kept of the emergence of the flies, as also of the flies that emerged in the other two battery-jars. In order to do this accurately it was necessary to remove the flies that had emerged in the jars each day. When, after a period of weeks or months as the case demanded, no more flies emerged, the sand in the jars was sifted and the remains examined. In most cases it was found that practically all the maggots had pupated and that flies had emerged from a very large percentage of the puparia. There were a few exceptions to this which will be dealt with below.

The life histories of the three species of sheep blow-flies will now be treated separately as they differ in certain essential details.

C. chloropyga.

Oviposition.—The female lays her eggs one at a time in quick succession in crevices in or under the meat. She prefers a carcass lying in the sun but the eggs are laid in the shade under the legs or in the cavities such as the mouth and nostrils. In the wool of live sheep the flies often work their way towards the skin until they are quite out of sight and sometimes get entangled and die in the wool.

The fly appears to be very excited, struggling in the wool, or walking rapidly about amongst the carrion with the ovipositor continually extending and contracting. When she has found a suitable place in which to deposit the eggs the telescopic hinder portion of her abdomen is entirely extended and the eggs pass down it. They slip out as if covered by some lubricant which soon dries and sticks them to the meat and to each other so that they form irregular masses.

The Egg.—The egg is of a pure creamy white colour and is 1.25 m.m. long and .32 m.m. wide, elongate oval in shape but slightly pointed at one end. At this end is the micropyle surrounded

by a shallow cup-shaped depression from the sides of which, on the dorsal surface, run two parallel lines or folds the whole length of the egg. The surface of the egg appears to be covered with a sheath of mucous like a fine membrane and in the surface of the chorion can be seen the fine hexagonal sculpturing so characteristic of insect eggs.

In many of the eggs the outside layer of mucous appears to crack and peel off showing the shining, transparent chorion beneath through which the embryo can be seen very clearly.

The cracking of this layer does not prevent the eggs from hatching normally.

Hatching of the egg.—The eggs take from eight hours to three days to hatch after being deposited, the time depending on the temperature and humidity of the surrounding atmosphere.

They become slightly darker in colour and when about to hatch the segmentation and mouthhooks of the embryo can be clearly seen as well as the trachae in its body which show up as silvery threads. In one case the segments of the embryo in an egg laid at 10 a.m., became visible at 7.30 on the following morning. Considerable movement of the body and mouthhooks was seen. This egg began to hatch at 8 a.m. and the maggot finally left the shell at 8.20. The mouth parts of the embryo can be seen moving round sideways and forwards as if to rupture the egg shell just before hatching takes place and the shell then tears open at the pointed end of the egg and splits along between the two parallel ridges. The movements of the larvae are intermittent and after breaking its shell it usually retracts and lies still in the egg for some minutes. It seems in no hurry to escape. The maggot is surrounded by a layer of liquid which helps it to slip out of the egg. It protrudes its body and retracts five or six times, each time pushing a little further out of the shell until finally its caudal extremity comes free and the maggot crawls down between the other eggs to the meat. When just out of the shell the maggot is very sticky and sticks to the other eggs or to the point of a dissecting needle. This is on account of the above-mentioned liquid which has partly dried. The hatching of the egg takes from about half a minute to thirty minutes and the egg shell then collapses and is left as a shrivelled white envelope.

Development of the Larvae.—The newly hatched maggot is pure white but already has the bands of minute black spines on the edges of the segments.

From a study of the spiracles of the maggot at different stages of its development it appears that the maggot moults three times before it becomes full grown.

Lowne (88) observed the first moult in the maggots of *Calliphora erythrocephala*, Meigen, two hours after hatching but in *Chrysomyia chloropyga* it takes place about four hours after hatching. At first each main tracheal trunk is divided at its end into two branches and each branch ends in a minute horse-shoe shaped disc in the centre of which is the actual opening. These discs are separate from each other. There is no appreciable increase in size of these discs until from four to six hours have elapsed when they become larger and they change in shape becoming elongate with the openings slot-shaped.

After about twelve hours another sudden change in the appearance of the discs takes place. They become larger again and the openings appear as typical slots in pairs at the end of each tracheal trunk surrounded by a slight peritreme. The peritreme becomes chitinized slowly so that the whole structure assumes a definite shape with two slots. After about thirty-six hours another moult takes place and the fully developed spiracle appears with three slots. At first the peritreme is indistinct and extends only partly round the spiracle but it gradually develops until when the maggot is full grown it appears as in plate VI, Figure 2.

Pupation.—When the maggot has finished feeding, it leaves the meat or the wool of the sheep and crawls about on the ground for a short time. It then buries itself in the sand, contracts in length, increases slightly in girth and lies still. It is then called the prepupa. If it is disturbed by being exposed to the light it will extend its body and become active again. Once the integument has begun to harden and turn yellow it cannot do this and pupation continues normally. The hardened integument containing the pupa is called the puparium. If a mass of maggots is left in an open dish they wriggle over each other and pupation is delayed, but eventually they will pupate although the first to do so are constantly tossed about by the others. The puparium first assumes a creamy yellow colour which gradually deepens through orange to a rich reddish-brown, finally becoming almost black. In the case of *C. chloropyga* puparia there is a tendency for grains of sand to stick to them. This is characteristic and often serves to distinguish them from the puparia of other flies.

Emergence.—When the fly is ready to emerge, the anterior end of the puparium is pushed open. The cap consists of the four anterior rings of the puparium and these break across its end forming, as it were, two concave doors which fold back to allow the fly to escape. The puparium is opened by the pulsating ptilinum which pushes out of the frontal suture and continues to expand and contract for half an hour or more after the fly has emerged. It is this pulsating ptilinum which enables freshly emerged flies to work their way up through the soil. When the fly emerges, its body is soft and of a pale greyish colour. Its wings are crumpled but its legs are well formed and it is able to walk rapidly. In a short time the cuticle hardens and assumes its normal green and blue colour and the wings become fully expanded.

Periods of Development.—By reference to the accompanying life history diagrams the development of the flies can be followed throughout an entire year.

The straight horizontal lines represent the lifetime of the successive generations of flies in the cages, the left-hand end of each line indicating the day on which the cage was set up and newly emerged flies put into it.

The vertical arrows above the horizontal lines represent egg masses laid by the flies on meat in the cages. The first three of these are longer than the others and are numbered I, II, III respectively. The egg masses represented by these were put under bell jars to hatch as explained above and the resulting larvae reared to maturity.

The numbers below the horizontal lines indicate the number of flies that died in the cages on successive days. The total number of males and females that were collected from each cage is given to the left. The day on which the last fly died in the cage is indicated by the right-hand end of the horizontal line. The short horizontal lines running to the right from the tips of the long vertical arrows represent the egg stage and the completion of the different stages of development is indicated by short vertical lines surmounted by capital letters. Thus the hatching of the eggs is indicated at H.

The approximate size of the resulting maggots on successive days is given in fractions of an inch to the right. The time at which the fully-fed maggots began to leave the meat is indicated at "A" and when they had all left the meat at "B".

The time at which the first maggots in each batch pupated is represented at P, and when they had all pupated at Q. The pupal stage is represented by a wavy line and the time at which the first flies emerged is indicated at E and the last at L, the period during which flies emerged being represented by a dotted line.

From the jar in which the first flies emerged, flies were put into the succeeding cage. This is indicated by a curved arrow running upwards.

Diagram No. 2. It will be seen at a glance that eight complete generations of *C. chloropyga* were reared during the period between November 13th, 1928, and December 9th, 1929. If maggots had developed from the eggs laid in cage No. 7 another generation would probably have been completed.

In the spring and autumn the flies lived in the cages 89 and 85 days respectively but in the heat of summer during February they lived only 23 days. They died off very quickly during the period February 8th to the 13th which was a period of the highest minimum temperature for the year and the maximum temperature rose to 94° Fahrenheit in the shade. These temperatures were accompanied by dry winds and dust storms.

Oviposition in the cages was very irregular. In December and January most eggs were laid and often in large masses, but at other times of the year it was difficult to obtain eggs in the cages.

During hot weather the eggs hatched in about twelve hours but in autumn they sometimes took three days to hatch. Usually, if they did not hatch within two days, they did not hatch at all. This was often the case in autumn. The maggots developed very rapidly in the middle of January, becoming full grown in three days whereas in May and June it took some of them twenty-five days to develop. This was the longest feeding period observed.

After leaving the meat some maggots pupated in two days. This was at the beginning of December, while in June some may not have pupated for nineteen days.

No maggots of *C. chloropyga* survived the winter as did those of *L. sericata*. No tendency was noticed of the maggots to delay their pupation for long periods. The shortest possible pupal period according to the diagram was three days during the summer months and forty-six days during the winter.

Only one batch of eggs laid in cage No. 6A developed and the maggots and puparia from these carried the species through the winter. All the maggots from this egg mass had pupated by June the 23rd.

On July 5th three flies emerged but died immediately on account of the cold, the maximum temperature being only 57° F., which was the lowest for the year.

On July 8th and 9th there was a fall of snow which was the heaviest that had been seen for seven years. After this the maximum temperature rose a little and on July 15th seven more flies emerged from these puparia and during the succeeding five days a few more emerged. This was very surprising as it was the middle of winter and the weather was still very cold and damp. Minimum temperatures continued to fall and reached 23° F. several times, so that by July 24th all these flies had died without laying eggs. Flies began to emerge again from these puparia on August 8th to carry on the generations. This was just after the minimum temperatures rose and did not fall again below 31° F. In these life history studies the maggots and puparia were dealt with in batches of fifty or more and records were not kept of the development of each individual maggot and puparium.

It is not possible therefore to give the exact periods of development because the rate of development of the individuals varied considerably and as they reached maturity a great amount of overlapping occurred.

In some cases all the maggots of a batch finished feeding and left the meat on the same day as at AB in jars No. 1 and No. 3 from cage No. 1. Here the shortest period between their leaving the meat and pupating was definitely two days.

In other cases there was an interval of fourteen days between the time when the first and the last maggots left the meat. In several cases all the maggots of the same batch pupated within one day of each other but in another case the last maggot pupated thirteen days after the first of the same batch. The pupal period also varied enormously so that flies sometimes emerged in a jar over as long a period of time as forty-one days. This was in the winter as mentioned above. In November females laid eggs four days after they emerged and continued to lay at intervals for forty-two days. In January and February they continued egg laying for fifty-eight days. In early spring, however, the first flies that emerged from the winter puparia did not commence egg-laying for forty days.

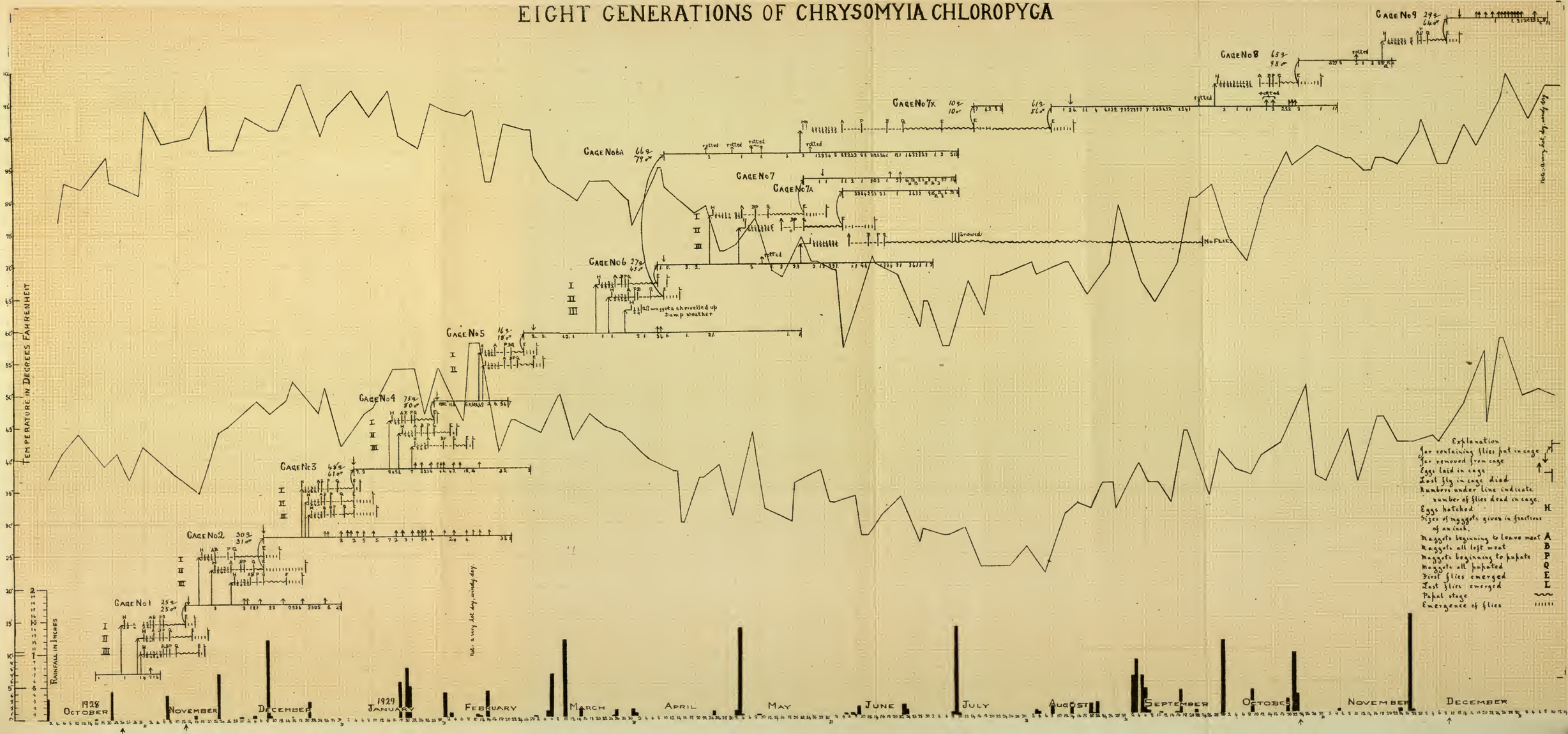
Number of Eggs Laid.

Several attempts have been made to determine the number of eggs the flies lay. In order to get a fair average, ten females with ten males were put into a cage and fed regularly on meat and sugar water. The meat was examined each day and the eggs on it counted under the microscope.

The first of these tests was started on March the 4th. The flies had just emerged in a battery jar. On the 24th the first batch of eggs was laid, but three females had escaped, so that there were only seven in the cage.

DIAGRAM No 2

EIGHT GENERATIONS OF CHRYSOMYIA CHLOROPYGA



Eggs were laid as follows:—

March 24th	1,542 eggs.
March 31st	1,499 eggs.
April 1st	123 eggs.
April 14th	5 eggs.
	3,169

The average per female was, therefore, 453.

In another case three females and five males were put into a cage just after emerging. This was on December 15th. On January 4th a large batch of 425 eggs was laid and on the 15th a smaller one containing 125 eggs. No more eggs were laid. The average per female in this case was 183 eggs.

A single pair of *C. chloropyga* flies was put into a cage immediately they emerged on January the 4th. The number of eggs laid were as follows:—

January 13th	87 eggs.
January 20th	57 eggs.
	144 eggs.

The male died on January 21st, but was replaced by another, and the female died on the 26th.

The number of eggs laid by the flies in the open is probably much greater than these figures would indicate.

In the autumn great difficulty was found in carrying on the generations and several cages of the same generation of flies were run simultaneously so that if one lot died out without laying eggs another might carry on.

Thus in cage No. 6 there were three batches of eggs that hatched. From two of these flies developed and were put into cages Nos. 7 and 7A. All these flies died without producing maggots and the species would have died out in the Insectary if another cage, No. 6A, had not been started with flies from the second batch of eggs laid in cage No. 5.

The rainfall is shown at the foot of the diagram by columns representing the amount of rain in inches on the day it fell. Apparently it does not affect the development of the flies directly, but it is unfavourable to the development of the eggs in cool weather because it encourages the growth of a mould which grows over them before they have time to hatch. After June 22nd no further eggs were laid.

The results of this study are most interesting when compared with the trapping records in a previous chapter, and we can now understand why this species has two periods of abundance in the year.

In December and January the flies lay large numbers of eggs and these hatch very readily so that the rate of reproduction is very great. In February the flies are killed off by hot, dry weather, but multiply again as autumn approaches. Their reproduction is soon checked, however, by conditions unfavourable to the hatching of the eggs.

Very cold weather, as was experienced during the period between the 17th and the 26th of July, also kills off the flies very rapidly.

L. sericata.

Periods of Development.—Diagram No. 3. The generations of *L. sericata* followed each other much more regularly throughout the year than those of *C. chloropyga* and nine complete generations were reared during the period between August 29th, 1925, and August 11th, 1926.

The longevity of the flies decreased regularly as the weather became warmer and they became more active and laid their eggs faster. In the autumn they became less active again and lived longer.

In early spring the maximum life of a fly was ninety-one days whereas in summer it was only thirty-five days. During January eggs were laid in cage No. 5 every day. Very hot weather particularly if dry and windy killed off the flies in the cages very rapidly. On February the 7th the temperature reached 100° F. in the shade and on this day the last surviving flies died in cages No. 3 and 4, twenty-one died in cage No. 5, leaving only four alive and eighty-four died in cage No. 6. The weather was very dry and dusty.

The laying and hatching of the eggs in the case of *L. sericata* is the same as described in the case of *C. chloropyga*.

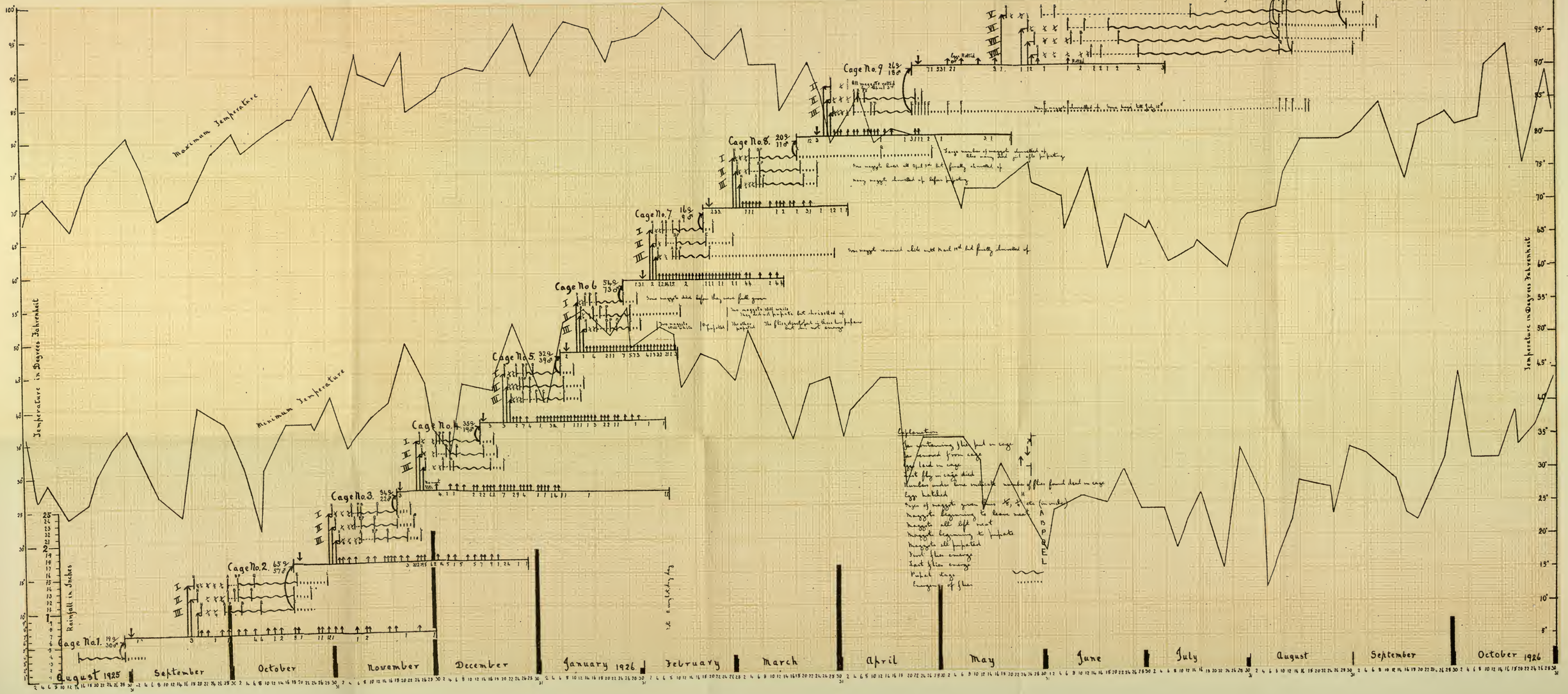
The incubation period is from eight hours to three days, depending on the time of year and all the eggs in a mass hatch within about two hours. The development of the larvae is essentially the same as that of *C. chloropyga* and is very rapid during summer, the shortest feeding period being only two days.

In autumn the maggots remained feeding in the meat for nineteen days. When fully fed the maggots leave the meat and after crawling over the surface of the ground for a short time burrow in and pupate. In one case as many as five days elapsed between the time the first and the last maggots left the same piece of meat.

The rate of development varies considerably in the later stages so that the flies originating from a single egg mass often emerge at intervals over a long period. Some of the maggots burrow into the soil within half an hour of leaving the meat while others have been found to crawl about on the surface for over three months. After burying themselves the maggots enter the prepupal stage and this also varies greatly in length: from a few hours to as long as sixty-eight days. If they are disturbed during this period they become active and crawl about until they can bury themselves again. They will sometimes pupate on the surface. The pupal period varies enormously in length, its minimum being only seven days in summer, while in winter it reaches its maximum of one hundred and fifteen days. Most of the flies hibernate as puparia buried in the soil. Some however, remain as white maggots in the prepupal stage until the spring. It is believed that some adult flies survive the winter in sheltered places for they may be caught in traps on warm days at any time of the year. They do not lay eggs at Grootfontein during the coldest period, from the middle of June to the end of August although in warmer parts of the country maggots are found in sheep at any time during the winter.

DIAGRAM Nº 3

Observations on the Life-History of the Sheep Maggot-Fly (Lucilia Sericata).



The fly therefore passes the winter in all its stages except the egg stage and breeds almost continuously throughout the year. During January and February eggs were laid in the cages every day. At this time of the year the flies begin to lay eggs five days after emerging from their puparia whereas in the spring they do not commence egg-laying until at least nineteen days have elapsed. The first egg masses laid are usually small. As the flies continue to lay the size of the masses increases while towards the end of the laying period the number of eggs decreases again. An average sized egg mass contains about one hundred and fifty eggs. As the flies age in the cages their wings become broken until they cannot fly. They become sluggish and the females usually die on the meat immediately after they have laid their last batch of eggs.

Number of eggs Laid.—From ten female *L. sericata* flies kept in a cage with ten males from the time of their emergence 8,757 eggs were obtained. Two of the females died however before any eggs were laid so that the average per female was 1,094. Salt (144) in England has recently obtained 1,883 eggs from one female. The number of eggs laid each day is shown in Table XVII.

TABLE XVII.

Date.	Condition of Flies.	Eggs Laid.
March 6th	Ten females emerged	—
„ 8th	One fly dead	—
„ 9th	One fly dead	—
„ 17th	Eight flies alive in cage	469
„ 18th	Flies active	1,313
„ 19th	Flies active	458
„ 21st	Flies active	639
„ 23rd	Flies active	106
„ 24th	Flies active	1,188
„ 25th	One fly dead	467
„ 26th	Flies active	54
„ 27th	Flies active	34
„ 29th	Flies active	1,405
„ 30th	Flies active	108
„ 31st	Flies active	113
April 2nd	Flies active	57
„ 3rd	Flies active	174
„ 4th	Flies active	163
„ 8th	Flies active	93
„ 9th	Flies active	699
„ 12th	Flies active	414
„ 13th	Flies active	117
„ 17th	Flies active	110
„ 19th	One fly sluggish; other flies active	27
„ 20th	One fly dead	23
„ 23rd	Flies active	224
„ 24th	Flies active	34
„ 29th	Flies active	182
May 2nd	One fly dead	—
„ 5th	Flies active	52
„ 7th	One fly dead	—
„ 9th	Flies well and active	34
	TOTAL	8,757

No more eggs were laid. The last of the four remaining females died on May 24th. In another case nine female *L. sericata* flies laid a total of 4,625 eggs.

We have no evidence that the flies are parthenogenetic. Ten females that had just emerged were put into a cage without males on April 27th. The first of these died on June 8th, and the last on July 2nd, but although the flies were supplied with meat and sugar water each day no eggs were laid.

The rainfall does not seem to affect the rate of development of the flies to any great extent but it affects the rate of reproduction by influencing the mortality amongst the eggs and pupae as described above.

Towards the end of the summer a high rate of mortality occurs amongst the larvae of *L. sericata* as has already been explained.

Underfeeding of Maggots.—It is well known that the size of flies depends on the amount of food they are able to obtain in their larval or maggot stage and Graham-Smith (58), has shown that the size of different individuals of the same species may vary very greatly. This we have often observed both in flies caught in the open and in flies reared in our Insectary. A large specimen of *L. sericata* may measure 11 m.m. in length while small ones often measure only 6 m.m.

On October 11th a large batch of *L. sericata* eggs was laid in one of our cages and by the morning of the 13th all these eggs had hatched. On the 15th the maggots were about $\frac{1}{4}$-inch long and ten of them were removed from the meat and put on to clean sand in a battery jar. A few were also killed and preserved in alcohol. Similar batches of the maggots were removed from the meat on the five following days, ten being put on to clean sand in a battery jar and a few in alcohol each day. By the 21st all the remaining maggots had completed their development and left the meat. They had all pupated by November 1st and on November 6th hundreds of normal flies emerged.

The maggots that were removed from the meat prematurely were kept under observation and the results are shown in Table XVIII.

It will be seen that in the first two jars no flies emerged. The maggots put into these jars were very small and about equal in size, those taken from the meat on October 16th having grown very little since the previous day. Between the 16th and 17th the maggots in the meat increased considerably in size so that those put into jar No. 3 were almost half an inch long. From ten of these three flies emerged. From the next batch, seven flies emerged and from those that were left in the meat six days, ten flies emerged from the ten maggots. From the fourth day the maggots increased in size at a uniform rate.

A study of the preserved material revealed the fact that the maggots had moulted between October 16th and 17th. The posterior spiracles of the maggots of the first two batches were only partly

TABLE XVIII.

Date.	Jar 1.	Jar 2.	Jar 3.	Jar 4.	Jar 5.	Jar 6.	Original Batch of Maggots.
October 13.....	All eggs hatched						
October 15.....	Maggots 2 days old; $\frac{1}{4}''$ long; removed from meat						
October 16.....	Maggots crawling on sand	Maggots 3 days old; $\frac{1}{4}''$ long; removed from meat					
October 17.....	All maggots shrivelled up	Maggots alive on surface of sand	Maggots 4 days old; $\frac{3}{8}''$ long; removed from meat				
October 18.....		All maggots shrivelled up	Maggots alive on surface of sand	Maggots 5 days old; $\frac{1}{2}''$ long; removed from meat			
October 19.....			2 pupated, 1 active; others shrivelled	9 active; 1 shrivelled up	Maggots 6 days old; $\frac{5}{8}''$ long; removed from meat		
October 20.....			3 formed shrivelled puparia	No change	All maggots active	Maggots 7 days old; $\frac{3}{4}''$ long; removed from meat	
October 21.....				No change	All maggots active	All maggots active	Maggots 8 days old; $\frac{3}{4}''$ long; left meat.
October 22.....				5 pupated	3 pupated	No change	
October 23.....				All pupated	8 pupated	No change	
October 24.....					All pupated	1 pupated	
October 25.....						3 pupated	
October 26.....						8 pupated	
Finally—	No flies emerged	No flies emerged	2 ♂ and 1 ♀ fly emerged; very undersized	3 ♂ and 4 ♀ flies emerged; well formed and almost normal size	4 ♂ and 6 ♀ flies emerged; normal in size.	2 ♂ and 6 ♀ flies emerged; 1 stuck halfway out of puparium; 1 shrivelled maggot	All maggots pupated by Nov. 1; hundreds of well-formed flies emerged

developed, having two slots in each spiracle. In the third batch the spiracles were fully formed, having three slots in each and very similar in appearance to those of full grown maggots, except that the peretreme was not fully chitinised. These maggots were therefore in their last instar. There was no obvious development of the spiracles in the subsequent batches of maggots.

There seems to be therefore a definite stage in the development of the maggot, probably the last moult, accompanied by a definite change in the structure of the posterior spiracles, after reaching which it is able to pupate and develop into a fly.

In the above experiment this stage was reached on the fourth day.

A larger proportion of flies emerged from the maggots the longer they were allowed to feed and the size of the flies increased as was expected.

It will be noticed that underfeeding has no effect on the sex of the resulting flies. This was further demonstrated by underfeeding another batch of maggots and examining the flies that finally emerged from them. In this case 15 flies emerged, 8 being males and 7 females.

C. albiceps.

Numerous attempts have been made to rear *C. albiceps* for generation after generation throughout an entire year as has been done with the other two species of sheep blow-flies, but without success. A great deal of information about the life history of this fly has been gathered in these attempts and a brief discussion of the work will be of interest.

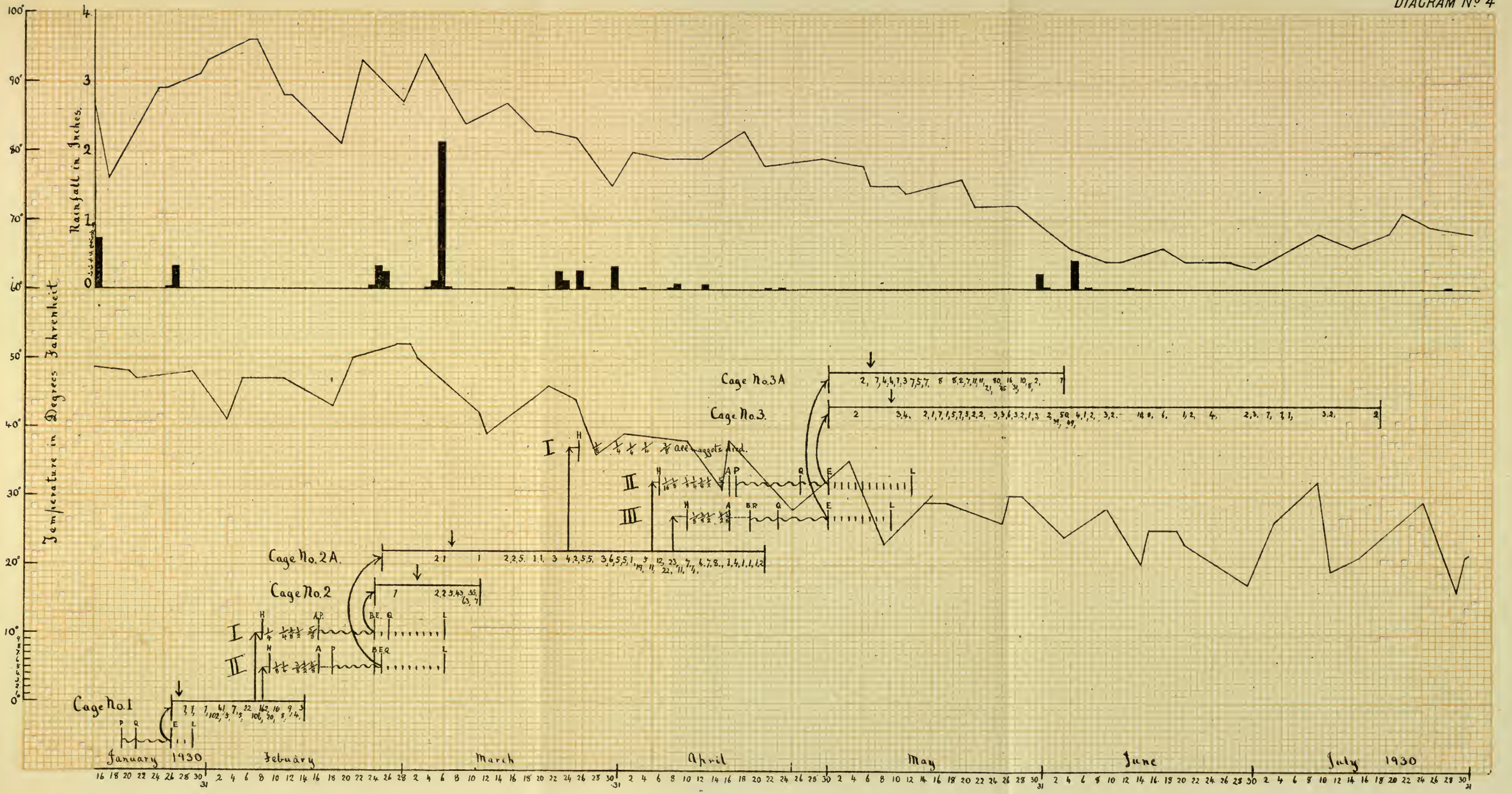
1. A large mass of eggs laid on meat in the Insectary at 11 a.m. on March 21st had hatched by the following morning. The maggots from these eggs were full grown by April 7th and some had pupated by the 8th. The maggots of this species tend to remain under the meat where they have been feeding and to pupate there. All had pupated by April 15th and on the 21st three flies emerged. The flies continued to emerge until April 24th but all died without laying eggs.

2. On March 14th a large mass of full grown maggots was taken from a carcase and put under sand in a battery jar. Flies began to emerge from these on the 25th and continued to do so until the 28th. They were put into a cage as they emerged and some of them lived until June 24th, a period of ninety-one days. Two large masses of eggs were laid on meat in the cage, one on May the 10th and the other on May 22nd, but none of these eggs hatched.

3. Another large mass of maggots was taken from a carcase in the veld on May 22nd and kept under sand in a jar. The sand was dampened from time to time and by June 5th all the maggots had pupated. The puparia were large and well formed. They were kept under observation each day until the following December, but no flies emerged. On December 27th many of the puparia were broken open

TWO GENERATIONS OF CHRYSOMYIA ALBICEPS.

DIAGRAM Nº 4



and no pupae were found in them. Nothing was found but a little whitish material dried against the insides of the puparia. The maggots had evidently died soon after pupation.

4. In another attempt a number of flies that emerged on October 29th from puparia collected under a carcase were put in a cage with meat and sugar water. By November 8th they had all died without laying eggs.

5 and 6. This was tried again in January, but the flies lived only from January 10th to the 14th, and again in February, when the flies lived from the 15th to the 23rd, in both cases without laying eggs. The last attempt has been more successful.

7. On January 15th a large number of full grown *C. albiceps* maggots were collected and these began to pupate on the 19th. (See Diagram No. 4.) Flies emerged on the 26th and were kept alive in a cage until February 14th. They laid two batches of eggs on February 7th and 8th respectively, and these all hatched in less than twenty-four hours. The maggots developed quickly and many from the first batch of eggs pupated on the 16th. Flies again emerged on the 24th, and were put in cage No. 2. These flies all died without laying eggs, although there were over a hundred flies in the cage. The maggots from the second batch of eggs began to pupate on the 18th and flies emerged from these on the 25th.

They were put in cage No. 2A. Three batches of eggs were laid in this cage on March 24th, April 5th, and April 8th respectively. The maggots from the first of these all died when about three-eighths of an inch long, but those from the other two batches developed and hundreds of well-formed flies emerged from their puparia on April 30th. Those from the second batch were put into cage No. 3 and those from the 3rd batch into cage No. 3A. All these flies again died without laying eggs, although in cage No. 3 some lived until July 18th, a period of seventy-nine days through the coldest part of the winter. Numerous attempts were made to induce the flies to lay eggs. In addition to the regular daily supply of fresh meat, bad meat in all stages of decay was exposed to them and also pieces of liver, kidney and entrails of sheep containing dung. The entrails seemed more attractive to the flies than the meat, but they did not lay eggs. Meat infested with the maggots of *L. sericata* was also exposed to the flies, and although they were strongly attracted to it, no eggs were laid. It is believed that in nature only a very small proportion of the flies survive the winter and in order to carry them through in the Insectary a larger number of flies will have to be used.

In the above experiment the flies would probably have laid eggs if they could have lived another two weeks as the weather becomes quite warm at the beginning of August.

The almost entire absence of *C. albiceps* in the spring and early summer is therefore due to the fact that the flies do not lay eggs in winter, that many of the eggs laid in autumn fail to hatch and that during the winter the maggots tend to die soon after forming the puparium.

PART III.

STUDIES OF THE SHEEP MAGGOTS.

Descriptions of the South African Sheep Maggots.

The maggots of *C. chloropyga* and *L. sericata* are very similar in appearance and it is difficult to distinguish them without microscopic examination.

Those of *C. albiceps* are easily recognised with the naked eye. The size of the full grown maggot is some guide, as also its colour, shape, and the appearance of its segments. This last depends again on the degree to which its armature of spines is developed.

The shape of the three species is very similar, being cylindrical pointed in front and gradually increasing in diameter towards the caudal end. The body is truncated behind obliquely, " so that the posterior extremity exhibits a concave surface which looks upwards and backwards and within which the posterior spiracles are situated." Lowne (88).

There are twelve visible segments, the nine posterior ones having a thickened anterior border forming footpads covered with very short recurved spines and sensory papillae. These aid the maggot in crawling and burrowing through the soil.

C. chloropyga.—The full grown maggot measures 18 mm. in length. It is of a dirty cream colour and its segmentation is well marked by the footpads covered with minute spines.

Its posterior end is surrounded by six pairs of tubercles, three above and three below, and a seventh pair on the ventral surface below the anus. These tubercles are more prominent than those of the *L. sericata* maggot but not so long as those of *C. albiceps.*

Its mouth hooks are as shown in Plate VII, Figure 1, and the posterior spiracles as in Plate VI, Figure 2. On the second segment from the front, on each side are the minute fan-shaped anterior spiracles.

L. sericata.—The full grown maggot of *L. sericata* is smaller than the other sheep maggots, being 16 mm. long and it is smoother and whiter in colour. The footpads are almost indistinguishable and its segmentation is, therefore, not so apparent. Its colour is usually a creamy white but it often has a pinkish tinge. Its mouth hooks are as shown in Plate VII, Figure 2, and the posterior spiracles as in Plate VI, Figure 4. These are quite distinct from those of the other species of sheep maggots as we should expect from the fact that *L. sericata* belongs to an entirely different genus from *Chrysomyia.*

The posterior end of the maggot is protected by tubercles arranged in the same way as those of *C. chloropyga* but they are much smaller.

C. albiceps.—The maggots of *C. albiceps* are quite distinct from the other sheep maggots. They are dark in colour and are covered with " spiniferous processes " bearing clusters of spines at their tips. On this account they are popularly known as " hairy maggots." The dark colour is produced by areas of minute dark studs which are very abundant on the dorsal and ventral surfaces.

EXPLANATION OF PLATE VI.

Fig. 1.—*Chrysomyia chloropyga* maggot.
Fig. 2.—Posterior spiracles of *Chrysomyia chloropyga* maggot.
Fig. 3.—*Lucilia sericata* maggot.
Fig. 4.—Posterior spiracles of *Lucilia sericata* maggot.
Fig. 5.—*Chrysomyia albiceps* maggot.
Fig. 6.—Posterior spiracles of *Chrysomyia albiceps* maggot.

SOUTH AFRICAN SHEEP MAGGOTS.

The spiniferous processes were arranged in longitudinal series, a pair of the longest running down the middle of the back, and four series on each side becoming smaller towards the ventral surface as shown in Plate VI, Figure 5. The length of the maggot is about 17 mm. and it is stouter than the other two. The mouth hooks are as shown in Plate VII, Figure 3, and the posterior spiracles as in Plate VI, Figure 6. The posterior end is well guarded by three pairs of tubercles above and three below while a seventh pair is situated on the ventral surface, posterior to the anus as in the other maggots. The tubercles are much longer and darker in colour than in the other two species.

1

2.

3.

B. Smit.

Mouth Hooks of South African Sheep Maggots.

EXPLANATION OF PLATE VII.

FIG. 1.—Mouth hooks of *Chrysomyia chloropyga* maggot.
FIG. 2.—Mouth hooks of *Lucilia sericata* maggot.
FIG. 3.—Mouth hooks of *Chrysomyia albiceps* maggot.

Habits.

The habits of the sheep maggots are chiefly concerned with feeding, self-preservation and migration from their feeding place prior to pupation.

Their method of feeding is most interesting. The maggots are able to produce a considerable amount of a slimy liquid from the mouth which has a strong digestive action and tends to liquify the meat in which they live, reducing it to a broth. Fat is not reduced and the maggots do not feed on it, so that a carcase that has been consumed by them and has dried somewhat, contains most of its original fat and burns very easily. The fact that the maggots throw out this digestive juice explains why patches of infested wool on sheep appear wet.

When feeding in meat, the maggots cluster in masses, lying closely packed next to one another with their pointed cephalic ends and bodies immersed in the semi-liquid meat broth, while their hinder ends, carrying the two caudal spiracles, project above its surface. These caudal spiracles can easily be seen as the maggot feeds. They are protected from immersion in the meat juice by the ring of projecting tubercles bordering the caudal end of the maggot so that a constant air supply is maintained.

In warm weather the maggots are very active and make a considerable noise when feeding in large masses. They need a plentiful supply of fresh air and if put in a closed bell jar will soon suffocate.

If thrown out of the meat they are apparently attracted to it again by smell for they will crawl up to it from a distance of twelve inches. If disturbed they become very active and wriggle furiously to get out of sight into the carrion. On living sheep the maggots frequently migrate through the wool from the crutch over the back leaving tracks that can be seen through the wool.

The maggots of *C. albiceps* are predacious on those of the other species of blow-flies and if thrown into a jar with them will destroy them. They will sometimes even attack each other. The hairy *C. albiceps* maggot curls itself about its smooth rival and pierces its tough integument with its sharp mouth hooks, after which it sucks out its body fluid leaving nothing but the shrivelled skin. The tubercles of the maggot help it in this operation and it holds so tightly to its prey that a large drop of white fluid is often pressed out of its victim when its skin is punctured.

In a carcase the different species of maggots keep more or less to themselves.

C. albiceps maggots seem to prefer feeding in or near the stomach contents of dead animals, after they have reached a certain stage in their development and will often remain in the dung for long periods finally pupating in it.

The maggots of the blow-flies feed very ravenously as if anxious to finish their meal as soon as possible and get away from the carcase to safety. When full grown the great majority leave the carcase entirely. Many bore down through the skin straight into the damp ground under the carcase, but most of them crawl away over the surface of the ground. They have been seen to crawl ten yards before burrowing into the soil beneath some stone or bush. Migration takes place chiefly at night.

If a mass of full-grown maggots is thrown out on to the ground they will all crawl away from the sunlight and seek shelter in dark crevices as quickly as possible. The depth to which the maggots burrow before pupation depends on the nature of the soil. In soft red soil many burrow four or five inches, although they are often found only an inch below the surface.

When a sheep infested with maggots is dressed with any of the standard carbolic dips the larger maggots immediately wriggle out of the wool and fall to the ground. They are not killed however and if they are caught in a basin and buried under a thin layer of soil, will pupate and the flies will emerge. If a piece of meat is overstocked with maggots to such an extent that they cannot possibly all develop, many of them will crawl away when about a quarter of an inch long and shrivel up without pupating. The others will continue to feed and although not able to attain their normal development will, nevertheless, pupate and produce flies.

The Effect of Maggots on Wool.

In most of the literature on sheep maggots it is stated that the maggots on an infested sheep rot the wool. It has now been found that this is not the case. Whitnall, working under the author's direction here, has studied this phase of the problem and the following is a brief summary of the results.

The maggots do not break through the skin of the sheep in the incipient stages of myiasis but live upon its surface, particularly in the folds which are often found beside the vagina of ewes. They work with their pointed, cephalic ends closely appressed to the skin and with their mouth hooks and the spines on the body cause constant irritation which soon sets up inflammation. The skin becomes red and sore. It also becomes very moist due partly to the digestive fluid thrown out by the maggots but also due to increased activity of the skin and the secretions of the sebaceous or sweat glands.

If wool is growing on the skin the moisture is soaked up by it and the wool is discoloured. It is this fact that has given rise to the wrong idea that the wool is rotted. The wool fibres are not corroded or eaten away either by the liquids produced or by the maggots themselves. A study of wool fibres amongst which maggots have been feeding on infested sheep reveals the fact that the substance of the fibres is not dissolved. The serrations show up under the microscope as do those of a normal fibre. This has been verified by Dr. Duerden, who is our South African authority on the structure of wool fibres.

There is a certain amount of felting and breaking due to the crawling of the maggots through the wool but this is purely mechanical.

It is well known that the wool falls off the skin where it has been badly infested with maggots. In this case the fibres are shed, not broken off.

This appears to be due to the inflammation set up by the maggots in the skin. The fibres come away with their "roots" or sheaths from the follicles so that the skin is left bare, but as soon as the skin becomes normal a new growth of wool begins. The shedding is similar to that seen when a sheep has a high fever and the re-growth of the wool to that of a sheep after recovery from such an illness.

It is difficult to understand on what the maggots feed while working on the surface of the skin. They grow very fast so that they must obtain a considerable amount of nourishment of some sort. Possibly it is provided by the secretions of the dermal glands of the sheep or by the yolk of the wool fibres. The maggots do not feed on dung caught in the wool.

Destruction of Maggots.

The question of destroying the sheep maggots is one which has received much attention from all workers on the sheep blow-fly problem, both from the point of view of killing the maggots in carrion and of discovering a suitable dressing for infested sheep. In this connection a large number of experiments have been carried out at Grootfontein.

Effect of Temperature.

In nature the maggots of blow-flies are sometimes subjected to extremes of temperature, but these extremes do not appear to harm them. Maggots have been left out in open evaporating dishes on the coldest winter nights when the temperature dropped to 20° F. and in the morning they appeared to be dead, but when the sun came up and warmed them they were as lively as ever. In the heat of summer they are able to crawl over the scorching surface of the ground which often rises to a temperature of 145° F. without being harmed.

In an attempt to ascertain what temperature blow-fly maggots can endure, the following experiments were carried out.

In Water.

Maggots in a small gauze basket were immersed in a large dish of water which was constantly stirred and gradually heated from below. A thermometer was placed with its bulb in the basket amongst the maggots. Two hundred maggots were used. As the water heated up the maggots became very lively until after a few minutes at a temperature of 122° F. (50° C.), they appeared to die with a convulsive jerk of the body. They were immediately cooled

in cold water and thrown on to dry earth in a jar. By the next day most of them had revived. They were crawling about and burrowing in the soil. Only a few had been killed. The others pupated and flies emerged as follows:—112 *L. sericata*, 43 *C. chloropyga*, 2 *C. marginalis*. A second batch of maggots was taken and the process repeated, raising the temperature of the water in the gauze basket to 123⁴/₅° F. (51° C.) before cooling. This apparently killed them all, but on the following day many had revived, and were crawling slowly on the surface of the soil. They pupated and the flies emerged as follows:—74 *L. sericata*, 1 *C. marginalis*.

The third batch was thrown out when the temperature of the water reached 125³/₅° F. (52° C.). These did not move at all or pupate. They were all killed.

On Dry Sand.

Maggots were placed on dry sand in a large evaporating dish and the sand heated gradually from below with an electric hot-plate. A thermometer was placed with its bulb half buried in the sand. The maggots became very lively as the sand heated up and began to die when the temperature reached 150° F. At 158° F. about half of them died and at 174° F. practically all were dead. Some that were able to crawl upon the bodies of the others lived a few minutes longer. The sand was cooled after reaching a temperature of 176° F. After two hours all the maggots were shrivelled up and had turned brown.

This experiment has been repeated several times with the same result. It does not show that the maggots can stand a temperature of 158° F., but merely that they may survive when crawling on a surface raised to this temperature. If stirred into the sand at this temperature they are immediately killed. The air above the hot sand is much cooler than the sand itself and circulates over it. Before death the maggots endeavour to reach up into this air in order to escape the heat.

In this connection it may be noted how difficult it is to kill all the maggots in a carcase by burning, unless the burning is done very thoroughly. This is partly due to the fact that meat is a bad conductor of heat, but also to the high resistance of the maggots.

Drowning in Pure Water.

Full-grown maggots cannot pupate in water, but eventually drown in it. In pure water they sink to the bottom but remain alive for a considerable time.

Seventeen jars were filled with water and into each, ten full-grown *C. chloropyga* maggots were thrown. After two hours those in jar No. 1 were thrown out on to dry sand, and those in succeeding jars at intervals of half an hour. The maggots in jar No. 17 were thus thrown out after immersion for ten hours. Most of the maggots burrowed into the sand and pupated and eventually flies emerged as shown in Table XIX.

TABLE XIX.

Number of Jars.	Time.	Number of Flies that Emerged.
1	2 hours	10
2	2½ ,,	10
3	3 ,,	10
4	3½ ,,	9
5	4 ,,	9
6	4½ ,,	9
7	5 ,,	9
8	5½ ,,	10
9	6 ,,	9
10	6½ ,,	6
11	7 ,,	5
12	7½ ,,	10
13	8 ,,	8
14	8½ ,,	6
15	9 ,,	8
16	9½ ,,	7
17	10 ,,	7

Immersion for ten hours therefore did not kill the maggots. They pupated and seventy per cent. of the flies emerged. A further series of the maggots was then taken and the first batch was thrown out of the water after eleven hours. Successive batches were taken out at intervals of an hour. The results are shown in Table XX.

TABLE XX.

Number of Jar.	Time.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies Emerged.
1. Check	Maggots not put in water	0	0	10
2	11 hours	1	2	7
3	12 ,,	0	1	9
4	13 ,,	0	0	10
5	14 ,,	0	0	10
6	15 ,,	0	0	10
7	16 ,,	0	1	9
8	17 ,,	0	1	9
9	18 ,,	1	0	9
10	19 ,,	0	3	7
11	20 ,,	0	0	10
12	21 ,,	1	1	8
13	22 ,,	0	1	9
14	23 ,,	3	1	6
15	24 ,,	2	2	6
16	25 ,,	0	7	3
17	26 ,,	0	6	4
18	27 ,,	0	7	3
19	28 ,,	1	5	4
20	29 ,,	0	5	5
21	30 ,,	0	6	4
22	31 ,,	0	6	4
23	32 ,,	0	8	2
24. Check	Maggots not put in water	0	1	9

Immersion for thirty-two hours therefore did not kill the maggots. They all pupated, but only two flies emerged from ten puparia.

Effect of Insecticides.—The resistance of full grown maggots to insecticides is most remarkable and is due to the very thick and impervious integument, to the fact that they do not feed when full grown and to the armature of the spiracles. The following experiments have shown how very resistant they are.

Powders.—Series A. Six batches of 50 maggots which were full grown but had not yet left the meat in which they were feeding, were thrown into glass beakers with 50 gms. of powdered insecticide. One batch was thrown into a clean beaker as a check lot. The maggots burrowed into the powders, becoming coated with them in a few minutes. The results were as shown in Table XXI.

This was merely a preliminary experiment but it serves to show how impervious the cuticular of these maggots must be. Mercury chloride (Corrosive sublimate) is one of the most poisonous of substances and yet some of the maggots lived with their bodies coated with it for 48 hours.

Sulphur apparently had no effect on the maggots and iron sulphate very little. Sodium fluoride and boracic acid prevented about half of the flies from emerging while borax killed 70 per cent. of the maggots on the seventh day and prevented all the flies from emerging from the puparia. It is interesting to note that borax has been recommended for killing house-fly maggots in manure heaps.

Further experiments were then carried out with the three species of sheep maggots separately. Batches of 50 full grown maggots were thrown into jars containing 25 gms. of the insecticides ground to a fine powder. The results are indicated in Tables XXII, XXIII and XXIV.

Conclusions.—Only eighty-two per cent. of the flies emerged from the check lot although all the maggots pupated. Many of the maggots in the jars had pupated in the powders at the end of twenty-four hours and most of the others were still alive.

Common salt had very little effect on the maggots. Borax only killed one maggot. The others pupated and thirty-one flies emerged.

Lime killed only one maggot, and twenty-nine flies emerged. Copper sulphate had practically the same effect. The insoluble arsenate of lead did not kill any maggots, but twenty-two pupae died in it and twenty-eight flies emerged. The more soluble arsenicals killed more maggots, arsenite of soda killing thirty-eight and the rest dying as pupae.

Potassium permanganate killed five maggots and all the rest as pupae.

Iodoform was very effective and the more volatile substances such as thymol, iodine and cyanogas killed all the maggots within a day.

TABLE XXI.

Powder.	First Day.	Second Day.	Third Day.	Sixth Day.	Seventh Day.	Fifteenth Day.	Finally.
Flowers of Sulphur	Maggots lively	Maggots lively	Maggots lively	3 maggots pupated; 1 dead	All others pupated	14 flies emerged	15 *C. chloropyga*; 34 *L. sericata* emerged.
Borax.....	Maggots lively	3 maggots dead, others lively	Other maggots lively	6 maggots pupated	Most maggots dying; 6 active	No flies emerged	34 maggots died; 16 pupated, but did not develop further
Sodium fluoride....	Maggots lively	3 maggots dead; others lively	7 maggots dead; others lively	Maggots pupating	43 maggots pupated	5 flies emerged	24 *L. sericata* emerged; 19 died in puparia; 7 maggots died.
Iron Sulphate.....	Maggots lively	Maggots lively	25 pupating; others lively	Almost all pupated; 5 dead	No change; 6 maggots dead	3 flies emerged	39 *L. sericata* emerged, 5 died in puparia; 6 maggots died.
Boracic Acid.....	Maggots lively	Maggots lively	Maggots lively	5 maggots pupated; others lively	25 maggots pupated; 5 dead	No flies emerged; 15 maggots dead	12 *L. sericata* emerged, 23 died in puparia; 15 maggots died.
Mercury Chloride...	25 maggots dead; others lively	43 dead, 5 still moving	All maggots dead	No change	No change	No flies emerged	No flies emerged.
Check.....	Maggots lively	Maggots lively	Maggots lively	A few pupated	45 pupated	All pupated; 22 flies emerged	12 *C. chloropyga*, 37 *L. sericata* emerged; 1 died in puparia.

NOTE.—The first day of the table is the day after the experiment was started.

TABLE XXII.

Series B. *C. chloropyga.*

Powder.	Effect during First Two Hours.	First Day.		Finally.		
		Maggots Pupated.	Condition of others.	Maggots Died.	Dead Pupae.	Flies Emerged.
Cheek.....	Maggots lively.	33	Crawling about	0	9	41
Flour.....	No effect.....	25	Crawling about	0	19	31
Calcium sulphate (Plaster of Paris)	No effect.....	5	Crawling about	0	7	43
Sodium Chloride (Common Salt)	No effect.....	40	Crawling about	8	5	37
Borax.....	No effect.....	45	Crawling about	1	18	31
Boracic Acid....	One maggot lying still; others lively	5	A few dying; others lively	7	12	31
Calcium Hydroxide (Lime)	No effect.....	45	Crawling about	1	20	29
Sodium fluoride	No effect.....	25	Active	3	18	29
Sulphur.....	No effect.....	25	Active	3	19	28
Arsenate of Lead	Two apparently dead; others lively	25	A few shrivelled; others lively	0	22	28
Copper Sulphate	No effect.....	25	Active	1	22	27
Paris Green.....	No effect.....	A few	Active	5	24	21
Pieric Acid.....	Maggots very active at first; a few dead after two hours	6	Many shrivelled up; Some still alive	26	18	6
Cooper's Powder Dip	Three dead after two hours; others lively	35	Most of others still alive	6	39	5
White Arsenic...	No effect.....	25	Still alive	6	41	3
Iodoform.....	No effect.....	12	Still crawling about	26	24	0
Arsenite of Soda	No effect.....	10	Still alive	38	12	0
Thymol.....	Maggots very active; 5 lying still after two hours	0	All maggots dead	50	0	0
Potassium Permanganate	Maggots stained black; ten lying still after two hours; others lively	10	A few dead; others crawling about	5	45	0
Iodine.....	Maggots stained brown; very active; all dead in two hours	0	All maggots dead	50	0	0
Cyanogas (Calcium Cyanide)	Several maggots dead in fifteen minutes; all dead in half-an-hour	0	All maggots dead	50	0	0

TABLE XXIII.

Series C. *L. sericata.*

Powder.	Effect during First Two Hours.	First Day.		Finally.		
		Maggots Pupated.	Condition of others.	Maggots Dead.	Dead Pupae.	Flies Emerged.
Cheek No. 1....	Maggots lively	1	Crawling about	0	0	50
Cheek No. 2....	Maggots lively	0	Active	1	0	49
Flour.....	No effect.....	0	Crawling about	0	3	47
Sodium Chloride	No effect.....	0	Crawling about	0	0	50
Borax.....	No effect.....	0	Crawling about	1	0	49
Boracic Acid....	No effect.....	0	Crawling about	1	0	49
Calcium Hydroxide	No effect.....	0	Crawling about	0	0	50
Sodium Fluoride	No effect.....	0	One dead, others moving	0	13	37
Sulphur.....	No effect.....	0	Crawling about	0	0	50
Arsenate of Lead	No effect.....	0	Active	0	3	47
Copper Sulphate	No effect.....	0	Three lying still; others crawling about	0	15	35
Paris Green.....	No effect.....	0	Three dead; others active	6	25	19
Pieric Acid.....	No effect.....	0	Five shrivelled up; others lying still	16	25	9
Cooper's Dip....	No effect.....	0	Crawling about	1	44	5
White Arsenic...	No effect.....	1	Crawling about	5	44	1
Iodoform.....	Maggots very active	0	Five dead; others sluggish	33	7	0
Arsenite of Soda	Maggots very active	0	Crawling about	0	50	0
Thymol.....	Maggots very active	0	Most dead; a few moving	50	0	0
Potassium Permanganate	No effect.....	1	Moving slowly	11	34	5
Iodine.....	Maggots very active	0	All dead	50	0	0
Cynogas.....	All still after ten minutes	0	All dead	50	0	0
Naphthalin.....	Maggots very active	0	Active	50	0	0

Conclusions.—Ninety-nine per cent. of the flies emerged from the checks.

The maggots did not pupate for several days but most of them were alive after twenty-four hours. On the whole they seemed more resistant than the maggots of *C. chloropyga* but they may have been a more vigorous lot to begin with as would be indicated by the emergence of flies from the checks.

The first five powders in the table had practically no effect on the maggots or their pupae and arsenate of lead had no more effect than flour.

Copper sulphate killed fifteen pupae.

The arsenicals again killed in proportion to their solubility, arsenite of soda killing fifty pupae.

From the potassium permanganate only five flies emerged and the volatile substances again proved most effective as in the case of *C. chloropyga*.

TABLE XXIV.

Series D. *C. albiceps.*

Powder.	Effect during First Two Hours.	First Day.		Finally.		
		Maggots Pupated.	Condition of others.	Maggots Died.	Dead Pupae.	Flies Emerged.
Check.....	Maggots very active	1	Active	2	12	36
Flour.....	No effect.....	2	Alive and active	0	2	48
Calcium Sulphate	No effect.....	4	Active	0	23	27
Sodium Chloride	No effect.....	2	Active	0	6	44
Boracic Acid....	No effect.....	0	All active	0	11	39
Calcium Hydroxide	No effect.....	2	Active	0	9	41
Sodium Fluoride	No effect.....	1	Active	0	21	29
Sulphur.....	No effects.....	2	Active	3	0	47
Arsenate of Lead	No effect.....	0	All active	0	12	38
Copper Sulphate	No effect.....	0	Active	0	7	43
Paris Green.....	No effect on maggots; powder sticks to them	0	All crawling slowly	3	45	2
Picric Acid.....	Irritates maggots and makes them very active	5	Active	0	35	15
Cooper's Dip....	No effect on maggots; they are covered with the powder	5	Active	5	39	6
White Arsenic...	No effect on maggots; they are covered with powder	3	Two shrivelled up; others moving	16	34	0

TABLE XXIV—*Continued.*

Powder.	Effect during First Two Hours.	First Day.		Finally.		
		Maggots Pupated.	Condition of others.	Maggots Died.	Dead Pupae.	Flies Emerged.
Iodoform.....	Irritates maggots and makes them very active	2	Alive, but sluggish	0	50	0
Thymol.....	Maggots very active	1	Most dead; one still moving	49	1	0
Potassium Permanganate	Stains maggots; they are very active	0	All shrivelled up	50	0	0
Iodine.....	Stains maggots; very active at first, but died within five minutes	0	All dead	50	0	0
Cyanogas (Calcium Cyanide)	Very active at first, but all dead within ten minutes	0	All dead	50	0	0

Conclusions.—In the case of *C. albiceps* only seventy-two per cent. of the flies emerged from the checks. This is a normal proportion for this species which is usually more difficult to rear than the other two. It must, however, be taken into account in analysing the results.

To some substances the maggots seemed more resistant than those of *C. chloropyga* and *L. sericata* but to others less.

Flour, common salt, boracic acid, lime, sulphur, arsenate of lead, and copper sulphate had no effect on them. Calcium sulphate seemed to prevent more of the flies from emerging than in the case of *L. sericata*. The soluble arsenicals were again effective and the volatile substances again killed the maggots.

Liquids.

The resistance of the sheep maggots to liquid insecticides is equally astonishing and has been demonstrated by the following series of experiments.

Series A.

Full grown *C. albiceps* maggots were used in batches of 50. They were put in small gauze baskets and dipped into the insecticides for various lengths of time. When taken out they were thrown on to dry earth in battery jars. The tops of the jars were covered with calico and the flies that finally emerged were counted.

The results are given in the Table XXV. It will be seen that immersion for 40 seconds in the carbolic solutions and paraffin had no effect on the maggots. The tar emulsion killed a fair proportion of them.

TABLE XXV.

Series A. *C. albiceps.*

Time Immersed.	Condition after 3½ Hours.	After 24 Hours.	Finally.
	Carbolic Acid—2 % Solution.		
10 seconds	All alive	2 dead	48 flies emerged.
20 seconds	All alive	2 dead	42 flies emerged.
30 seconds	All alive	10 dead	25 flies emerged.
40 seconds	One dead	3 dead	47 flies emerged.
	Carbolic Sheep Dip—1 % Solution.		
10 seconds	All alive	5 dead	42 flies emerged.
20 seconds	2 dead	5 dead	43 flies emerged.
30 seconds	2 dead	2 dead	40 flies emerged.
40 seconds	All alive	2 dead	44 flies emerged.
	Paraffin, Pure.		
10 seconds	4 dead	4 dead	43 flies emerged.
20 seconds	3 dead	5 dead	37 flies emerged.
30 seconds	All alive	3 dead	45 flies emerged.
40 seconds	1 dead	2 dead	47 flies emerged.
	Stockholm Tar Emulsion.		
10 seconds	All alive	2 dead	45 flies emerged.
20 seconds	3 dead	12 dead	35 flies emerged.
30 seconds	All alive	12 dead	25 flies emerged.
40 seconds	25 dead	36 dead	12 flies emerged.

Series B.

Batches of 50 full grown *L. sericata* maggots were immersed for one minute in various insecticides. They were then thrown on to clean, dry sand in battery jars as described above. After the flies had all emerged in the jars, the sand was sifted. The results are shown in Table XXVI.

TABLE XXVI.

L. sericata.

Liquid.	Condition after 24 Hours.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Paraffin, pure	1 maggot dead; 3 pupated; others burrowed into sand.	10	4	36
Benzine, pure	12 maggots have burrowed into sand; others dead on surface.	46	3	1
Turpentine, pure	1 maggot dead on surface; others in sand.	10	4	36
Carbolic acid, 5 %	All maggots buried themselves in sand.	5	3	42
Raw linseed oil, pure	1 maggot dead on surface; others in sand.	4	6	40
Carbon-bi-sulphide, pure	All maggots dead within ten seconds.	50	0	0
Tincture of iodine	All maggots buried themselves in sand.	5	7	38

TABLE XXVI—*Continued.*

L. sericata.

Liquid.	Condition after 24 Hours.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Glycerine, pure....	1 maggot dead on surface ; others in sand.	11	4	35
Formalin, 2 %....	3 maggots dead ; others in sand.	10	10	30
Tobacco extract, 1 % nicotine	2 maggots dead ; others in sand.	4	4	42
Methyl alcohol, 95 %	5 maggots dead ; 3 crawling on surface ; others in sand.	13	2	35
Ethyl alcohol, 95 %	All maggots buried themselves in sand.	8	5	37
Carbolic sheep dip. 5 %	All maggots buried themselves in sand.	7	3	40
Amyl salicylate, pure	5 maggots crawling on surface ; others in sand.	9	4	37
Methyl salicylate, pure	10 maggots dead on surface ; others in sand.	25	2	23
Pyridine, pure.....	Maggots all dead when thrown on to sand.	50	0	0
Potassium permanganate, 5 %	All maggots buried themselves in sand.	18	6	26
Corrosive sublimate, 5 %	7 maggots crawling on surface ; others in sand.	43	6	1
Sodium fluoride, 5 %	2 maggots crawling on surface ; others in sand.	22	6	22
Stockholm tar, pure	2 maggots pupated ; others dead on surface of sand.	48	2	0
Check No. 1.....	Maggots all buried themselves in sand.	5	7	38
Check No. 2.....	Maggots all buried themselves in sand.	9	5	36

Conclusions.—Only 74 per cent. of the maggots in the check jars developed into flies and it is noticeable that from several of the dipped batches of maggots a larger percentage of flies emerged. The most effective insecticide was carbon-bisulphide which killed all the maggots immediately. Pyridine killed them within a minute and Stockholm tar killed them all but two, within five minutes.

Benzine killed 46 maggots. Four pupated and only one fly emerged, while corrosive sublimate killed 43 maggots, seven pupated and one fly emerged.

Sodium fluoride solution killed about half of the maggots as did methyl salicylate. The alcohols had no effect on them and many other common substances such as paraffin, turpentine, tobacco extract and carbolic sheep dip also had no effect on them in one minute.

SERIES C.

Batches of 50 full grown *L. sericata* maggots were dipped in liquid mixtures in this case, for five minutes. They were then thrown out on to clean sand in battery jars as before.

The results are shown in Table XXVII.

TABLE XXVII.

L. sericata

Liquid.	Condition after 24 Hours.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Cotton-seed oil, 90 %, carbolic acid 10 %	Most maggots dead; a few moving about slowly.	48	0	2
Cotton-seed oil 50 %, fish oil 50 %	All maggots buried themselves in sand.	0	0	50
Fish oil, pure.....	All maggots buried themselves in sand.	3	10	37
Cotton-seed oil 90 %, oil picis 10 %	All maggots buried themselves in sand.	0	1	49
Cotton-seed oil 90 %, fusil oil 10 %	About half of maggots dead; others crawling about.	23	12	15
Cotton-seed oil 90 %, pyridine 10 %	Most of maggots dead; a few just moving.	43	5	2
Cotton-seed oil 90 %, solignum 10 %	All maggots buried themselves in sand.	1	0	49
Stockholm tar, pure...	All maggots apparently dead on sand.	49	0	1
Cotton-seed oil, pure..	All maggots buried themselves in sand.	0	8	42
Cotton-seed oil 50 %, paraffin 50 %	All maggots buried themselves in sand.	0	4	46
Solignum, pure.....	All maggots dead on surface of sand.	50	0	0
Glycerine, pure.....	All maggots buried themselves in sand.	0	6	44
Common salt (saturated solution)	All maggots buried themselves in sand.	18	0	32
Copper sulphate (saturated solution)	All maggots buried themselves in sand.	5	8	37
Acetic acid, 33 %.....	All maggots buried themselves in sand.	0	7	43
Check No. 1.....	All maggots buried themselves in sand.	0	0	50
Check No. 2.....	All maggots buried themselves in sand.	0	2	48

Conclusions.—98 per cent. of the *L. sericata* flies in the check jars emerged.

Carbolic acid, 10 per cent. in oil, killed forty-eight maggots and pyridine forty-three.

Pure Stockholm tar killed forty-nine and solignum fifty.

Fusil oil 10 per cent. in oil killed twenty-three maggots and only fifteen flies emerged.

Common salt solution killed eighteen maggots but copper sulphate solution killed only five after immersion for five minutes.

The other mixtures had practically no effect on the maggots.

SERIES D.

In this series full grown *L. sericata* maggots were used in batches of fifty. They were dipped in a sweetened solution of arsenite of soda such as is used for locust destruction.

The solution was made up by dissolving 4 grams of sugar and 2 grams of arsenite of soda powder in one pint of water (.3 per cent. solution).

The maggots were left in the solution for different lengths of time and the results are shown in Table XXVIII.

TABLE XXVIII.

L. sericata.

Period of Immersion.	Effect on Maggots.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
30 minutes....	All maggots alive and moving slowly when taken out.	2	3	45
40 minutes....	A few apparently dead; others moving.	5	9	36
50 minutes....	All lying still when thrown out.	9	3	38
60 minutes....	A few moving when taken out; others apparently dead.	4	10	36
Cheek.....	Maggots normal.	0	4	46

Apparently this solution is too weak to have any appreciable effect on the maggots.

SERIES E.

In this series full grown maggots of *C. chloropyga* were used and the method was the same as in Series C. The results are shown in Table XXIX.

TABLE XXIX.

Series D. *C. chloropyga.*

Liquid.	Effect on Maggots.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Benzine, pure.....	A few maggots moving after one minute; all dead after two minutes.	50	0	0
Benzene, pure.....	All dead after about ten seconds	50	0	0
Carbon-bisulphide, pure	All dead after ten seconds.	50	0	0
Paraffin, pure.....	Ten maggots moving when taken out; others apparently dead.	50	0	0
Pyridine, pure.....	All dead after four minutes.	50	0	0
Solignum, pure.....	Very active when first thrown in; all dead after two minutes.	50	0	0
Formalin, 4 %.....	All moving slightly when taken out.	50	0	0
Amyl salicylate, pure..	All apparently dead after three minutes.	50	0	0
Maglo.....	All apparently dead after one minute.	50	0	0
Bedwell's eucalyptus oil	All apparently dead after half a minute.	50	0	0
Cooper's Healing oil...	Very active when first thrown in; all dead after three minutes.	50	0	0
*Mixture D.....	All apparently dead after one minute.	50	0	0

* NOTE.—"Mixture D" is made up as follows:—Cotton-seed oil, 45 parts by volume; petrol, 40 parts by volume; pine tar oil (oil picis), 10 parts by volume; carbolic acid, 5 parts by volume.

TABLE XXIX—*Continued.*

Liquid.	Effect on Maggots.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Methyl salicylate, pure	All moving slowly when taken out.	48	1	1
Turpentine, pure.....	Very active when first thrown in; twenty moving when taken out.	46	4	0
Acetic acid, 33 %.....	All moving slowly when taken out.	43	7	0
Corrosive sublimate, 4 %	All moving freely when taken out.	41	9	0
Pure oil picis.....	Ten maggots moving when taken out; others apparently dead.	39	10	1
Lime sulphur (concentrated)	Did not seem to effect them; all alive when taken out.	39	11	0
Carbolic acid 5 %, Cotton-seed oil 95 %	All alive and normal when taken out.	37	11	2
Paraffin 50 %, Cotton-seed oil 50 %	All lively when taken out.	36	9	5
Pyridine 10 %, Cotton-seed oil 90 %	All lively when taken out.	31	16	3
Carbolic dip 5 % (in water)	All lively when taken out.	31	10	9
Copper sulphate (saturated solution)	All lively when taken out.	30	19	1
Tobacco extract (7·4 % nicotine)	Ten maggots apparently dead; others moving slowly when taken out.	27	21	2
Ethyl alcohol (absolute)	All moving slowly when taken out.	27	21	2
Stockholm tar 10 %, Cotton-seed oil 90 %	Very active when first thrown in; all moving slowly when taken out.	22	23	5
Fish oil, pure.....	All lively when taken out.	20	28	2
Methyl alcohol 95 %...	All moving freely when taken out.	19	29	2
Tincture of iodine.....	Five maggots apparently dead when taken out; others normal.	7	39	4
Solignum 10 %, Cotton-seed oil 90 %	All maggots normal when taken out.	12	30	8
Oil picis 10 %, Cotton-seed oil 90 %	All lively when taken out.	11	34	5
Potassium permanganate 5 %	All moving freely when taken out.	10	36	4
Picric acid (saturated solution)	All moving freely when taken out.	9	18	23
Crude glycerine.....	All maggots normal when taken out.	9	31	10
Sodium fluoride 5 %..	Five maggots apparently dead; others normal when taken out.	7	29	14
Sodium chloride (saturated solution)	All maggots normal when taken out.	5	13	32
Fusil oil 10 %, Cotton-seed oil 90 %	All maggots very active when taken out.	3	24	23
Raw linseed oil.....	Three maggots apparently dead; others normal when taken out.	2	8	40
Concoction of peach leaves	All maggots normal when taken out.	1	40	9
Check No. 1.....	Normal.	6	20	24
Check No. 2.....	Normal.	0	29	21

Conclusions.—Only 45 per cent. of the *C. chloropyga* flies emerged in the check jars so that the results of this series of experiments are not very reliable. The volatile substances were effective in killing all maggots and paraffin also killed them all. The three proprietary dressings were also very effective. Of the substances commonly recommended for dressing infested sheep, copper sulphate killed thirty of the maggots, but salt solution had practically no effect.

Series F.

The syrup containing arsenite of soda and molasses which is supplied by the Government for the destruction of locusts was then tested, but in this case the *C. chloropyga* maggots were left in it much longer.

Four strengths were used. The standard strength as used for locusts was first tried. This is one part of the syrup to fifty parts by volume of water.

Maggots left in this solution for one hour were all alive when taken out. They burrowed into soil in a battery jar but failed to pupate. No flies emerged from them. After an hour and a half two maggots out of fifty appeared dead but the others crawled about. Four pupated and from these one fly emerged.

After two hours about half of the maggots appeared dead. The others burrowed into the soil but failed to pupate. No flies emerged.

The poison was then tried at double, treble and four times the standard strength.

All the maggots were alive after one hour's immersion in the double strength poison, and when thrown on to clean soil two pupated. No flies emerged, however.

After an hour and a half forty-seven out of fifty maggots were alive. They crawled about on the soil but shrivelled up without pupating. After two hours twenty out of fifty were alive, but none of these pupated.

None of the maggots dipped into the stronger poison pupated. After an hour in the poison at three times normal strength all the maggots were alive but died soon afterwards. The same was true after an hour and a half. After two hours only ten out of fifty maggots moved. After an hour in poison at four times the normal strength forty-seven out of fifty maggots were alive. After an hour and a half thirty were alive and after two hours only ten out of fifty were able to move.

Series G.

In this series full grown maggots of *C. albiceps* were used, fifty in a batch. They were dipped in the liquids for five minutes as in Series C. The results are shown in Table XXX.

TABLE XXX.

C. albiceps.

Liquid.	Effect on Maggots.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Benzine, pure.....	All dead after ten seconds.	50	0	0
Benzene, pure.....	All dead after ten seconds.	50	0	0
Carbon-bisulphide.....	All dead after five seconds.	50	0	0
Paraffin, pure.....	All moving slowly when taken out.	16	20	14
Pyridine, pure.....	About half of maggots moving slowly when taken out.	50	0	0
Solignum, pure.....	Most maggots moving when taken out; a few dead.	26	23	1
Formalin, 40 %.....	Most maggots moving when taken out; a few dead.	50	0	0
Amyl-salicylate, pure..	Most maggots moving when out; a few dead.	26	15	9
Maglo.....	All dead after one minute.	50	0	0
Bedwell's eucalyptus oil	Most maggots moving when taken out: a few apparently dead.	15	3	32
Cooper's healing oil....	All moving when taken out.	5	14	31
Mixture D.....	A few moving when taken out; others apparently dead.	16	21	13
Methyl-salicylate, pure	About half moving when taken out; others apparently dead.	50	0	0
Turpentine, pure.....	About half moving slowly when taken out; others apparently dead.	30	9	11
Acetic acid, 33 %.....	Most maggots moving when taken out; a few apparently dead.	19	13	18
Corrosive sublimate, 4 %	All moving when taken out.	3	27	20
Pure oil picis.....	About twenty dead when taken out; others moving slowly.	30	11	9
Lime sulphur (concentrated solution)	All moving when thrown out.	5	23	22
Carbolic acid 10 %, Cotton-seed oil 90 %	Two apparently dead when taken out; others moving.	11	15	24
Paraffin 50 %, Cotton-seed oil 50 %	All alive when taken out.	15	11	24
Pyridine 10 %, Cotton-seed oil 90 %	All moving slowly when taken out.	12	30	8
Carbolic dip 50 % in water	Most maggots apparently dead when taken out.	0	1	49
Copper sulphate (saturated solution)	All moving when taken out.	1	4	45
Tobacco extract (7·4 % nicotine)	Most maggots moving when taken out; a few apparently dead.	23	14	13
Ethyl alcohol (absolute)	Most maggots moving when taken out.	7	11	32
Stockholm tar 10 %, cotton-seed oil 90 %	All moving when taken out.	4	23	23
Fish oil, pure.....	All moving when taken out.	0	21	29
Methyl alcohol 95 %...	Most maggots moving when taken out.	9	10	31
Tincture of iodine.....	All moving when taken out.	24	26	0

TABLE XXX—*Continued.*

Liquid.	Effect on Maggots.	Finally.		
		Dead Maggots.	Dead Pupae.	Flies.
Solignum 10 %, cotton-seed oil 90 %	All moving when taken out.	1	13	36
Oil picis 10 %, cotton-seed oil 90 %	All alive when taken out.	9	11	30
Potassium permanganate 5 %	All alive when taken out.	5	23	22
Picric acid (saturated solution)	Few lying still; others alive when taken out.	0	0	50
Crude glycerine.....	All alive when taken out.	1	4	45
Sodium fluoride 5 %...	Few lying still; others moving when taken out.	4	13	33
Sodium chloride (saturated solution)	All alive when taken out.	0	0	50
Fusil oil 10 %, cotton-seed oil 90 %	All moving, some very slowly, when taken out.	48	0	2
Raw linseed oil.....	All alive when taken out.	1	13	36
Check No. 1.....	Normal.	0	0	50
Check No. 2.....	Normal.	0	0	50

Conclusions.—In this series the results were far better than any of those given above as 100 per cent. of the *C. albiceps* flies emerged in the check jars. This is remarkable for the species and shows that conditions were ideal at the time of the experiment.

The maggots showed a remarkable resistance to many of the liquids but the volatile substances again proved very effective. Paraffin killed sixteen maggots out of fifty, but fourteen flies emerged, while solignum killed twenty-six maggots and only one fly emerged.

From maggots dipped in Bedwell's Eucalyptus oil and Cooper's healing oil thirty-two and thirty-one flies emerged respectively.

Turpentine killed thirty maggots but eleven flies emerged. Carbolic sheep dip, 5 per cent. Copper sulphate solution, salt solution and picric acid had practically no effect on the maggots.

Summary.

On the whole the three species of sheep maggots appear to be about equally resistant to insecticides. Their resistance is remarkable though the volatile substances are very effective in destroying them.

As a result of the above experiments and much practical experience, Mixture D is now recommended to farmers for use as a dressing on sheep infested with maggots. (See *Farming in South Africa*, August, 1930.) It has been thoroughly tested on infested sheep during the last two seasons and has given excellent results. Owing to the fact that it contains a large proportion of petrol, it is cheap and effective in killing the maggots. It also spreads well through the wool. The cotton seed oil soothes the skin of the sheep and helps to allay inflammation. The oil picis acts as a repellent

in keeping the flies from re-infesting the sheep and the carbolic acid destroys harmful bacteria. It is remarkable how quickly a badly infested sheep will recover after treatment with this mixture.

Disposal of Carcasses by Burying.

In most articles on blow-fly control the disposal of carcasses by burying is recommended. In practice it usually happens that farmers do this after the carcasses have become infested with the eggs or maggots of the sheep blow-flies. In the Karroo area, where the ground is very hard and dry, carcasses are not often buried more than two or three feet below the surface and often they are simply thrown into a " sluit " and covered with a foot or so of loose soil.

In order to show how ineffective such methods are in preventing the flies from breeding, the following experiments were carried out:

Burying of Full-grown Maggots.

A. A large pit, 36 inches deep was dug in the hard limy soil, and into this were thrown several carcasses. Four days afterwards they were heavily infested with maggots and large numbers of flies were swarming about the pit. On the eighth day most of the maggots were full grown and were beginning to leave the carcasses which by this time had subsided so that they lay right at the bottom of the pit. The pit was then filled in and all the soil, that had been taken out, was heaped over it. On the sixteenth day a large gauze cage was put over the whole pit. It was noticed that hundreds of maggots had worked their way to the surface of the soil and many had pupated just below the surface. On the twenty-first day the flies began to emerge and in a few days the inside of the cage was black with them. These were mostly *C. chloropyga* with a few *L. sericata* and *C. marginalis* amongst them. It was estimated that over 45,000 flies emerged under the cage.

B. A similar experiment was next carried out but in this case the pit was 48 inches deep. Two sheep, weighing together 74 lb., were thrown into it. By the thirteenth day the carcasses were swarming with maggots and some were beginning to leave the meat. The pit was filled in as before, the heap of loose soil extending well over the edges of it. On the nineteenth day heavy rain fell and the soil was thoroughly wetted and sank considerably. The cage was put over on the twenty-fourth day and a large number of maggots and puparia were noticed in the soil about an inch below the surface.

On the twenty-ninth day the flies began to emerge and continued to do so until the thirty-fifth day. They were identified and counted as follows:—

C. chloropyga	145
L. sericata	615
C. croceipalpis	47
M. domestica	55

When the carcasses were thrown into the hole, they were cut open. The house flies evidently developed in the dung exposed amongst the entrails.

Burying of Full-grown Maggots with Lime.

C. Lime is said to prevent flies from breeding, so in order to test the truth of this, a third pit was dug; in this case again 36 inches deep. A small carcass, weighing 30 lb. was thrown into it and on the sixth day it was swarming with full-grown maggots, a few of which had left the meat. 10 lb. of white, slaked lime were thrown over the carcass and the bottom of the pit, covering it to a depth of about half an inch. The maggots became very active and tried to get away from the lime by wriggling under the carcass. The contents of the pit were then carefully covered with soil and the pit filled up. By the following morning many maggots had come to the surface. A cage was put over the pit on the thirteenth day and by the thirtieth day the flies had all emerged. They were identified and counted as follows:—

C. chloropyga	107
L. sericata	19
C. albiceps	1

Burying of Eggs and Immature Maggots.

D. Nine glass measuring cylinders, 12 inches high and 2 inches in diameter were used in this experiment and into the bottom of each a small piece of meat was placed with a batch of fly eggs on it. The cylinders were numbered consecutively and No. 1 was immediately filled to the top with loose, red soil, previously sifted. The other jars were filled with soil in succession, one each morning, and the following records were kept of the development of the eggs, maggots and flies.

Cylinder No. 1.

Eggs on meat buried under 12 inches of soil.

1st Day. Eggs hatched and the maggots feeding in the meat.

2nd Day. Maggots about $\frac{1}{8}$ inch long.

3rd Day. Maggots developing slowly.

4th Day. Maggots about $\frac{1}{4}$ inch long.

5th Day. Maggots about $\frac{3}{8}$ inch long. The meat was at the bottom of cylinder but the meat juice had moistened the soil about half way up. Some of the maggots were in this wet soil half way to the surface, others still in the meat.

7th Day. The moisture had risen two thirds the height of the cylinder. Much of it was apparently produced by the maggots which were half an inch long. Some were right at the surface of the soil; others at various depths and many still in the meat.

11th Day. Many maggots still in the meat; others in all parts of cylinder. They had not increased in size.

17th Day. Maggots still about ½ inch long; some pupated at the surface of the soil; others in the soil at various depths, still active.

24th Day. Many *L. sericata* flies emerged; they were very much undersized, some being only 3.5 m.m. long.

Finally 34 undersized *L. sericata* flies emerged. There were hundreds of undersized puparia and freshly emerged flies dead in the soil near the top of the cylinder.

Cylinder No. 2.

The cylinder was filled with soil twenty-four hours after meat and eggs had been put into it. The eggs had all hatched and the maggots were feeding under the meat.

The next day the maggots were almost ⅛ inch long and still feeding in the meat.

By the fifth day the maggots were almost ½ inch long. The meat juice had risen in the soil half way up the cylinder. A few maggots had come to the surface but most of them were still feeding in the meat. They developed faster and to a larger size than those in Cylinder No. 1. On the 11th day two undersized flies emerged.

Finally 123 *L. sericata* flies emerged. They were undersized, but not as small as those in Cylinder No. 1. The puparia were in all parts of the cylinder and there were a few freshly emerged flies dead about four inches from the surface.

Cylinder No. 3.

The maggotts were ⅛ inch long, feeding under the meat when the cyclinder was filled up with soil.

On the fifth day the maggots were almost full grown and feeding in the meat at the bottom of the cylinder. The meat juice did not rise more than two inches in this jar.

By the seventh day a few maggots had left the meat. They appeared full grown. Flies did not emerge until the 24th day. They were slightly undersized, but larger than those in Cylinder No. 2.

Finally 164 *L. sericata* flies emerged. They were only slightly undersized. There were a few dead maggots in the meat at the bottom of the cylinder.

In Cylinder No. 4 243 flies developed and they were normal in size. The soil was put over the maggots on the third day after the experiment was started. Large numbers of flies developed in all the other cylinders and they were all full sized specimens.

In this experiment the chief factor appeared to be the air supply. The maggots had no difficulty in crawling about in the soil and getting to the meat. They worked their way into it and liquefied it. They were then able to feed on the meat juice but when the soil about the meat became wet the air supply of the maggots was cut off and they were forced to come to the surface before they were full grown. There they pupated producing undersized flies. Those that were able to develop normally for the first three days completed their feeding in the meat.

The meat that was left exposed a longer time contained less moisture when buried and therefore the maggots in the cylinders that were filled on and after the third day obtained better conditions for development, even after the soil was put over them.

It seems remarkable that the eggs were able to hatch under a foot of soil, but it is believed that they would be able to hatch under a much greater depth if they were not crushed. The danger of burying carcasses is therefore apparent even if they appear to be free from maggots, for the eggs of the sheep blow-flies are usually laid in the natural cavities of a carcass such as the mouth and nostrils where they are not easily seen and burying such a carcass does not necessarily prevent the flies from breeding in it.

Burying of Full-grown Maggots at greater depths in Tubes.

E. Full-grown *L. sericata* maggots were buried under loose sifted soil at the bottom of a glass tube five feet long and three quarters of an inch in diameter.

Twelve maggots were taken on each occasion and the results were as follows:—

Batch No. 1.—Under three feet of soil. After two hours the maggots had worked their way 18 inches up the tube. After seven hours they were almost at the surface and before 24 hours had elapsed, they had reached the surface and burrowed down into the soil again.

Batch No. 2.—Under four feet of soil. After 17 hours all the maggots had reached the surface of the soil and burrowed down into the soil again.

Batch No. 3.—Under five feet of soil. After 22 hours all the maggots had reached the surface of the soil and burrowed down into the soil again. In this case five adult flies finally emerged in the tube. The others did not emerge from their puparia. The puparia were found about half way down the tube. Fabre (38) described an experiment with the maggots of *Sarcophaga carnaria* in which the maggots were placed on the surface of soil in a tube similar to the one described above and in which they burrowed three feet into the soil. This is far deeper than they would go in nature or in a wider tube. He believed it was due to some form of light stimulus and the same may have been true in our experiments.

F. Full-grown maggots of *L. sericata* were buried at the bottom of tubes built up of circular tins the tops and bottoms of which had been carefully removed. Ten such tubes were used. The bottom tin of each tube was set in a layer of cement at the bottom of a pit four feet deep and the tubes were built up on these tins. The tins with their tops and bottoms removed, were fastened one above the other with adhesive insulation tape wrapped round the joints between them. Each tin was four inches high. The shortest tube, called No. 1, was made up of nine tins and was, therefore, three feet high. No. 2 was built up of ten tins, and so on as shown in the tables. No. 10 tube was thus composed of eighteen tins and was six feet high. Fifty active maggots were then put into each tube.

Tubes built up of circular tins used in the burying experiments. The tubes are shown surrounded with soil in the pit, with their tops covered with calico to retain the flies that emerged.

The tubes were all filled to the top with loose, sifted red soil and their tops were covered with calico. Soil was banked around them in the pit as shown in the accompanying photograph to support them and also to keep the maggots under the same conditions as if buried in the ordinary way.

The object of using tubes made up in sections as above described was to make possible the recovery of the remains of maggots, puparia or flies from each section separately, and thus ascertain what actually happened in each case. This was done by removing the earth from around the tubes and the insulation tape from the joints. Flat pieces of sheet metal were slipped in between the tins and the soil in each sifted separately through fine gauze.

The tubes were set up on April 30th. By the next day the soil in them had settled a little and in No. 1 tube the surface appeared to have been disturbed by maggots. One was found just under the surface but none were on the surface of the soil. Heavy rain fell during May. On July 2nd three live *L. sericata* flies emerged at the top of tube No. 7.

The tubes were finally taken down on August 26th and the results are shown in Table XXXI.

In this first attempt a great many of the maggots could not be accounted for. This was due to the fact that the flies developed very slowly, it being winter time, and the experiment had to be left undisturbed for nearly four months. During this time the remains of many of the maggots and flies that died in the soil became mouldy and disintegrated.

In spite of this a number of very remarkable facts were brought to light. In tube No. 10 thirteen normal flies emerged from 50 maggots that were buried under six feet of soil and in several of the other tubes, although not so deep, a remarkable proportion of the flies arrived at the surface. In most cases these flies emerged from puparia near the surface, showing that the maggots had worked their way up through a considerable depth of soil.

Many maggots pupated right at the bottom of the tubes and there were many empty puparia there showing that the flies are able to emerge successfully under a great depth of soil.

At the bottom of tube No. 4 a fully developed fly was found with its wings expanded and its characteristic colouring perfect. In tube No. 7 a live healthy maggot was found at the bottom under 5 feet of soil. It had been there for 120 days.

The results were not so satisfactory as was hoped, owing to the disapperance of many maggots as explained above and to the fact that water found its way into some of the tubes, causing variations in the conditions. The experiment was, therefore, repeated in the summer and with much better results. Development was rapid and it was not necessary to leave the maggots, puparia and flies in the soil for so long a period. Conditions were also kept uniform throughout the series of tubes. The tubes were set up and surrounded with soil on January 2nd.

In this case full grown maggots of *C. chloropyga* were used and fifty maggots were carefully thrown into each tube. A shaft of sunlight reflected down the tubes from a small mirror revealed the maggots all well and active. Sifted red soil was then poured into each

tube until it was full and the tubes covered with calico. The soil was dry and friable. The tubes were uncovered and taken to pieces on January 20th, and the number and sex of the flies in the top of each tube recorded. The soil in each tin was then sifted and the position of the remains of maggots, puparia and flies noted.

A check lot of fifty maggots was kept under half an inch of soil in a battery jar. All these maggots had pupated on January 5th and on the 11th 19 males and 11 females emerged. Flies commenced to emerge in the tops of the tubes on the 12th. The results are shown in Table XXXII.

Some maggots were again unaccounted for but not many. It is believed that they became mouldy and disintegrated. A number of the empty puparia were found adhering to the inside of the tins with the open end upwards although a few flies had emerged downwards.

In this experiment only five perfect flies reached the surface from maggots buried under six feet of soil, but in the shorter tubes many more did so, 43 reaching the surface in Tube No. 1.

Eight maggots pupated under six feet of soil and the flies escaped from their puparia, but we cannot say how far up the tube they climbed. At least two of them must have reached the seventh tin from the bottom, i.e. they climbed two feet four inches. In tube No. 9 three of the flies must have climbed to this height. In tube No. 8 one must have climbed three feet four inches and in tube No. 6 three feet eight inches. In tube No. 5 at least eight flies climbed four feet four inches right to the surface and in tube No. 4 twenty-eight flies climbed four feet.

In tube No. 3 at least thirteen flies reached the top from the puparia at the bottom, in tube No. 2 two, and in tube No. 1 four. These figures are obtained by subtracting the number of crumpled flies in the soil from the number of empty puparia at the bottom of the tubes. Some of the flies in the soil might have emerged from puparia higher up the tubes, however, and in that case the number of flies which reached the surface from the bottom would be greater. For a knowledge of the distance through which the maggots are able to crawl upwards we have only to note the positions of puparia. In tube No. 10 two maggots ascended five feet before pupating.

In tube No. 9 one maggot came to the surface but then died. Five pupated four feet four inches from the bottom.

In tube No. 8 two came to the surface and pupated there, while in the shorter tubes many pupated between four and twelve inches from the surface.

It is surprising to note how many maggots pupated right at the bottom of the tubes. Practically all the maggots pupated and their puparia were accounted for. Many of the flies that emerged were not accounted for. This was because the hard, tough puparia were more resistant to decomposition than the flies. Near the bottoms of tubes Nos. 1 and 2 fully coloured flies were found with their wings expanded as if they had hardened in sunshine. From the majority of puparia flies emerged but many of these died in the soil, probably from exhaustion and were found as shrivelled grey teneral flies with their wings still crumpled. Many flies in this condition were also found dead on the surface of the soil, particularly in tubes Nos. 2, 3, 4, 6, 7 and 9.

TABLE XXXI.—*Lucilia seriata* Maggots buried under different depths of loose soil.

Tins.	Ht.	Tube No. 10.	Tube No. 9.	Tube No. 8.	Tube No. 7.	Tube No. 6.	Tube No. 5.	Tube No. 4.	Tube No. 3.	Tube No. 2.	Tube No. 1.
		7 ♂ flies 6 ♀ flies									
18	72	8 empty puparia 1 crumpled fly	No flies								
17	68	4 empty puparia	1 dead pupa	No flies							
16	64	—	—	—	2 ♂ flies 1 ♀ fly	13 ♂ flies 6 ♀ flies 1 crumpled fly 2 empty puparia 1 live pupa					
15	60	1 empty puparium	—	—	3 empty puparia 1 live pupa						
14	56	1 empty puparium	—	—	—	18 empty puparia 3 live pupa	3 ♀ flies				
13	52	—	—	—	—	—	3 empty puparia 2 live pupa	10 ♂ flies 16 ♀ flies 1 crumpled fly			
12	48	—	—	1 empty puparium	—	—	—	21 empty puparia 1 dead pupa 1 dead maggot	1 ♂ fly		
11	44	—	—	—	—	—	—	2 empty puparia	1 empty puparium	23 ♂ flies 18 ♀ flies	17 ♂ flies. 10 ♀ flies. 2 empty puparia. 3 live pupae. 1 live maggot.
10	40	—	—	—	—	—	—	—	—	34 empty puparia 1 live pupa 1 live maggot 1 crumpled fly 1 dead maggot	
9	36	—	—	—	—	1 crumpled fly	—	3 empty puparia	—	6 empty puparia	22 empty puparia. 7 live pupae. 2 live maggots.
8	32	1 empty puparium 1 live maggot	1 empty puparium	1 dead maggot	—	—	1 empty puparium	2 empty puparia	—	—	1 empty puparium. 2 live maggots. 1 dead maggot.
7	28	—	—	—	—	2 empty puparia 1 dead maggot	—	1 empty puparium 1 crumpled fly	—	—	—
6	24	—	—	—	—	1 empty puparium	—	—	—	—	—
5	20	—	—	—	1 live maggot	—	—	1 empty puparium	—	—	1 empty puparium.
4	16	—	—	—	—	1 crumpled fly	—	—	—	—	—
3	12	1 empty puparium	—	—	1 live maggot 1 empty puparium	1 empty puparium	—	1 live maggot	—	—	1 empty puparium.
2	8	2 empty puparia	—	1 crumpled fly 1 empty puparium	2 empty puparia	—	2 empty puparia	2 empty puparia 2 crumpled flies	—	—	—
1	4	1 empty puparium 1 dead pupa	2 empty puparia	—	1 live maggot 3 empty puparia	—	1 live maggot	8 empty puparia 1 fly 1 crumpled fly	1 empty puparium	3 empty puparia 1 dead pupa 3 crumpled flies	1 empty puparium. 2 live pupae.

TABLE XXXII.—*Chrysonyia chloropyga* Maggots buried under different depths of loose soil.

Tins.	Ht.	Tube No. 10.	Tube No. 9.	Tube No. 8.	Tube No. 7.	Tube No. 6.	Tube No. 5.	Tube No. 4.	Tube No. 3.	Tube No. 2.	Tube No. 1.
—	—	2 ♂ flies 3 ♀ flies 2 crumpled flies									
18	72	4 crumpled flies	3 ♂ flies 8 crumpled flies 1 dead maggot								
17	68	1 crumpled fly	1 crumpled fly	5 crumpled flies							
16	64	2 empty puparia 1 crumpled fly	—	1 empty puparium 1 dead pupa 2 crumpled flies	9 crumpled flies						
15	60	2 empty puparia	1 crumpled fly	—	6 crumpled flies	1 ♂ fly 6 ♀ flies 13 crumpled flies					
14	56	2 empty puparia 1 crumpled fly	5 empty puparia 1 crumpled fly	2 crumpled flies	8 crumpled flies	7 crumpled flies	6 ♂ flies 3 ♀ flies				
13	52	3 crumpled flies	2 empty puparia 2 crumpled flies	2 empty puparia 1 crumpled fly	6 empty puparia 1 dead pupa 2 crumpled flies	2 empty puparia 1 crumpled fly	4 crumpled flies	15 ♂ flies 14 ♀ flies 2 flies (partly coloured) 8 crumpled flies			
12	48	3 crumpled flies	3 crumpled flies	5 crumpled flies	2 empty puparia 3 crumpled flies	10 empty puparia 2 crumpled flies	1 crumpled fly	2 ♀ flies	8 ♂ flies 12 ♀ flies 14 crumpled flies		
11	44	7 crumpled flies	2 crumpled flies	2 crumpled flies	2 crumpled flies	2 empty puparia 2 crumpled flies	3 empty puparia	—	1 fly 3 crumpled flies	12 ♂ flies 14 ♀ flies 12 crumpled flies	
10	40	3 crumpled flies	1 empty puparium 1 crumpled fly	6 crumpled flies	5 crumpled flies	1 empty puparia 1 crumpled fly	1 empty puparia	—	8 empty puparia	1 crumpled fly	22 ♂ flies. 21 ♀ flies.
9	36	1 crumpled fly	2 empty puparia	2 empty puparia 6 crumpled flies	1 empty puparium 3 crumpled flies	1 empty puparium 3 crumpled flies	1 crumpled fly	—	2 empty puparia 1 crumpled fly	2 empty puparia	1 empty puparia.
8	32	1 empty puparium 4 crumpled flies	4 crumpled flies	2 empty puparia 7 crumpled flies	2 crumpled flies	1 empty puparium 2 crumpled flies	1 dead pupa 1 crumpled fly	1 empty puparium 1 crumpled fly	4 empty puparia 1 crumpled fly	7 empty puparia 2 crumpled flies	19 empty puparia. 1 crumpled fly.
7	28	7 empty puparia 3 crumpled flies	2 crumpled flies	2 empty puparia	2 empty puparia 3 crumpled flies	2 empty puparia	6 crumpled flies	—	1 empty puparium	2 empty puparia 2 crumpled flies	1 dead pupa. 2 crumpled flies.
6	24	2 empty puparia 1 dead pupae	3 crumpled flies	2 empty puparia 1 crumpled fly	1 empty puparium 2 crumpled flies	—	1 empty puparium 4 crumpled flies	1 empty puparium 1 crumpled fly	1 crumpled fly	—	1 empty puparium.
5	20	3 empty puparia 3 crumpled flies	3 empty puparia 1 dead pupa 2 crumpled flies	2 crumpled flies	2 empty puparia 1 crumpled fly	1 empty puparium	4 crumpled flies	1 empty puparium	1 empty puparium	—	8 empty puparia. 1 crumpled fly.
4	16	4 empty puparia	6 empty puparia 3 crumpled flies	6 empty puparia	8 empty puparia	1 empty puparium	1 empty puparium	1 empty puparium	1 empty puparium	1 empty puparium 2 fully coloured flies	2 empty puparia.
3	12	4 empty puparia	5 empty puparia	2 empty puparia 1 dead pupa	6 empty puparia	4 empty puparia	1 empty puparium 1 dead maggot	4 empty puparia 1 dead pupa	1 empty puparium	4 empty puparia 2 fully coloured flies	4 empty puparia. 1 fully coloured fly.
2	8	9 empty puparia 2 dead pupae	9 empty puparia 1 crumpled fly	1 empty puparium	5 empty puparia	7 empty puparia 5 dead pupa	2 crumpled flies	6 empty puparia	2 empty puparia 1 dead pupa	19 empty puparia 2 dead pupa	4 empty puparia.
1	4	8 empty puparia	14 empty puparia	23 empty puparia 6 dead pupae	15 empty puparia 1 dead pupa	9 empty puparia 3 dead pupa 1 crumpled fly	36 empty puparia 5 dead pupa 2 crumpled flies	32 empty puparia 3 dead pupa	20 empty puparia 6 dead pupae 1 dead maggot	11 empty puparia	9 empty puparia. 1 dead pupa.
Maggots unaccounted for.....		3	0	0	0	1	0	0	2	7	0

Maggots Escaping from Jars or Tanks.

The maggots of the sheep blow-flies, in addition to being able to work their way through soil, are able to squeeze themselves into small crevices and exert considerable pressure, while their bodies are greatly flattened. In this way they are able, by working together in batches of fifty or more, to force their way under the edges of heavy bell jars standing on sheets of plate glass in the Insectary. The edges of the jars are ground to fit the glass but are slightly rounded inside the flat ground portion, so that the maggots are able to wedge themselves under them, but when it is realised that one of these jars weighs eleven pounds, the burrowing power of the maggots appears marvellous.

It is a very difficult matter to confine the maggots, partly on account of their burrowing habits and also on account of their ability to crawl up smooth surfaces, and it is for this reason that many maggot traps fail. The maggots crawl by means of their mouth hooks and the rows of minute spines on the underside of the body segments. On dry surfaces they are not able to grip very well and will roll down a smooth surface tilted at less than 45°, but if the surface is wet and particularly if it is covered with the slimy secretion from their mouths, they can easily crawl up perpendicular surfaces such as the sides of a glass jar. They will often crawl all over the inside of a bell jar of which the inside is wet and slimy. If thrown into water at the bottom of an iron tank many of the maggots will succeed in crawling up its smooth perpendicular sides to a distance of two feet and so escape if the sides of the tank remain damp. The same has happened in a cement tank three feet deep containing a little water which was used for the disposal of carcasses. The inside of this tank when new and clean could not be scaled by the maggots except at night when it became damp with dew. Even then the maggots did not seem able to climb more than half way up it, but as it became covered with their excretion the maggots climbed up more easily until finally the whole inside of the tank became coated with a moist, greasy substance which enabled the maggots to escape from the tank at any time of the day or night.

In order to prevent maggots crawling up smooth surfaces, it is only necessary to place them on a little dry soil on which they will clean and dry themselves. If the inside of the containing vessel is then clean and dry they cannot escape from it.

The maggots can also use their mouth hooks for tearing their way through calico covers of jars or the plugs of cotton wool at the mouth of tubes in the same way as they tear their way through meat, wool or living flesh.

Emergence of Flies through Soil.

In order to show more definitely that the flies can emerge from their puparia at great depths and work their way through the soil to the surface the following experiment was carried out.

Glass tubes, four feet long and two inches in diameter, were used, and one end of each was securely plugged with a tight fitting cork. The tubes were fastened vertically in a frame with their closed ends downwards and into each were put ten *Lucilia cericata* puparia. These puparia were obtained by allowing full grown maggots to pupate normally under half an inch of sand.

The tubes were then filled to different heights with loose red soil as shown in the table. The soil was first sifted to make sure that no other puparia were present. The tops of the tubes were covered with calico to retain any flies that emerged through the soil. After eleven days the flies began to emerge and the total number that finally emerged in each tube is shown in Table XXXIII.

It will be noticed that all the flies emerged through two feet of soil and nine through two feet three inches. Greater depths than this seemed to prevent the flies from escaping, although out of ten puparia, two came successfully through three feet six inches of soil.

TABLE XXXIII.

Number of Tube.	Depth of of Soil.	Number of Perfect Flies that Emerged.
	Inches.	
1	6	10
2	8	8
3	10	10
4	12	9
5	14	10
6	16	7
7	18	8
8	20	10
9	22	10
10	24	10
11	27	9
12	30	7
13	33	0
14	36	3
15	39	2
16	42	2

A further experiment was conducted as follows:—

From a jar in which *C. chloropyga* flies were emerging twenty-four of the flies were selected. They had only just come out of their puparia and were still grey and soft with their wings crumpled up. They were very active. Twelve of these were immediately buried under six inches of loose red soil, previously sifted, and twelve under a foot of this soil. Some of the flies could be seen in the soil through the sides of the glass jars in which they were buried. They began to work up through the soil and in ten minutes had raised themselves about an inch in both jars. After twenty-four hours five flies had reached the surface from under a foot of soil but four of these were

dead, their bodies being still grey and their wings crumpled up. The other fly coloured up and expanded its wings. In the other jar there were also five flies at the surface after twenty-four hours, the flies having worked their way through six inches of soil. Three of these were dead and two alive, but all were still grey with their wings crumpled.

After three days the jars were examined for the last time and the soil was carefully removed to see what had happened to the flies. The results were as follows:—

Jar containing twelve inches of soil.

Five dead flies at the surface, one normal and four shrivelled.
One shrivelled fly just below the surface.
Two shrivelled flies half way down the jar.
One shrivelled fly three inches from the bottom of the jar.
Three shrivelled flies at the bottom of the jar.

Jar containing six inches of soil.

Five dead flies, all shrivelled, at the surface.
One shrivelled fly just below the surface.
Six shrivelled flies at the bottom of the jar.

Flies that have hardened, assumed their normal colour and have expanded their wings cannot work their way through soil at all. If a mature fly is buried under half an inch of loose sand it will not be able to move and will die in the position in which it is placed under the sand. In the experiment described above it is probable that all the flies buried had begun to harden and that their ability to push through the soil depended on the extent to which this had taken place. The ptilinum of each fly was seen to pulsate just before it was buried, but in some cases it did so more vigorously than in others.

Summary.

From the above results it appears that it is very difficult to confine the sheep maggots in jars or traps and that burying a carcass containing blow-fly eggs or maggots is an unsatisfactory method of destroying them. Burying a carcass containing full grown maggots to a depth of six feet is not effective in destroying all the maggots, for some of them will complete their development and the flies will emerge. The maggots are able to burrow through the soil to the surface from a depth of six feet, but in many cases they pupate far below the surface and the flies emerge from the puparia normally. Newly-emerged flies are able to work their way up through at least four feet four inches of loose soil, but mature flies cannot move if buried under half an inch of loose sand.

PART IV.

PARASITES OF BLOW-FLIES.

Importance.

A great deal of interest has been taken in the question of blow-fly parasites and much information has been published on the subject. At one time, in Australia, it was hoped that the distribution of parasites would solve the sheep blow-fly problem there and much time and money was spent on distributing them

A special insectary was built and the parasites reared in enormous numbers, over fifty thousand parasitized fly puparia being sent out to sheep farmers per week. The parasite in this case was *Mormoniella vitripennis*, Walker. The results were disappointing and the attacks of sheep blow-flies increased in spite of this work.

A great many species of blow-fly parasites are known, particularly in Australia, but most of them appear to be very scarce and of little economic importance.

These parasites attack the flies at various stages of their development, some attacking the maggots and others the puparia. Their effectiveness depends largely on their habits in this respect.

It is recognized that the use of parasites in the case of the sheep blow-flies may prove an inexpensive and lasting remedy, and that if the numbers of flies can only be slightly reduced by these parasites their introduction and spread is justified provided they do not attack other flies that are beneficial.

List of Species.

A list of the better known blow-fly parasites and their hosts may here be useful for future reference.

Cynipids.

Various species belonging to the genera *Diranchis*, *Kleidotoma* and *Figites* which emerge from the puparia of flies.

Note.—The chapter on blow-fly parasites was written as part of the present paper, but was read before the Zoological Section of the British Association for the Advancement of Science at its recent meetings in Johannesburg. It has since been published in the *South African Journal of Science*, but is included here, with plates of the two parasites studied, in order to make this paper complete. The parasites have not been studied further since the chapter was written. No more *Alysia manducator*, Panz., adults emerged during the summer of 1929-30, and the parasite has, therefore, died out and will have to be reimported before further observations can be made. It appears that a very small proportion of maggots were actually parasitised. The *Alysia manducator* larvae developed in the puparia of these, spun their cocoons, and pupated, but the adults failed to emerge, presumably owing to unfavourable climatic conditions.

Proctotrupids.

Several species of *Ancurrhychus* and two of *Trichorpia*.

* *Paraspilomicecus froggatti*, Johnston and Tiegs. Attacks Lucilia in Australia.

* *Hemilexomyia abcupta*, Dodd. Attacks *Ophyra nigra* and *Calliphora villosa*, Desv., in New South Wales and has also been reared from *Musca domestica*, Linn., and *Pollenia stygia*, Fabr.

Braconids.

Alysia manducator, Panz., attacks many species of flies in Europe and Australia.

Aphaereta cephalotes, Hal.

Aspilota fuscicoenis, Hal.

Aspilota nervosa, Hal.

Ichneumonids.

Phygadeuoa speculator, Thoms. and *Atractodes bicolor*, Gravenh., have been reared from puparia of *Calliphora erythrocephala*, Meigen, in England.

Chalcids.

Melittobia acasta attacks many species of flies.

Dibrachys carus oviposits in the puparia of *Muscina stabulans*, Fall., and *Phormia azurea*.

Mormoniella vitripennis, Walker (*Nasonia brevicornis*, Ashm.). Attacks all common species of blow-flies and some Sarcophagids.

Muscidifurax rapor.

Neocemnus leucarthros, Thoms.

Spalangia hirta, Hal.

* *Spalangia muscidarum*, Richardson. Attacks the common house-fly, blow-flies and flesh-flies in Australia.

Spalangia grotiusi, Girault. Attacks blow-flies in Australia.

Australeucaytus giraulti, Johnston and Tiegs. (*Stenosterys fulroventcalis*, Dodd). Attacks all common sheep flies in Australia.

Chalcis calliphoeae, Frogg. Attacks maggots of *Anastellorhina augur*, Linn., in Australia.

Chalcis dipterophaga. Girault and Dodd. Found on blow-fly puparia in Australia.

Chalcis fonscolombei. Attacks maggots of Sarcophagids and Lucilia in Australia.

* *Dichinus sarcophagae*, Frogg. Attacks Sarcophaga aurifrons and all common sheep flies in Australia.

* *Pachycrepoideus dubius*, Ashm. Attacks house flies in United States and Australia.

NOTE.—Those marked * are said to be too scarce to be of any economic importance in Australia, where they are found.

When considering the introduction of any of these species it must be remembered that the greatest caution is necessary. Our knowledge of the habits of most of them is still very meagre and will have to be much increased before any such steps are taken. The range of hosts in many cases is very wide and it is very likely that by introducing them we should harm certain of our beneficial flies such as the dipterous parasites of our terrible brown locust, or the beneficial Syrphids.

It should also be noted that these parasites have apparently not controlled the sheep blow-flies in the countries of their origin. This would be expected from the nature of the case, the flies possessing such enormous powers of reproduction and man having already upset the balance of nature by producing the modern Merino sheep.

Mormoniella vitripennis, Walker.
(Nasonia brevicornis, Ashmead.)

* As far as is known, the only parasite of blow-flies in South Africa is the small Chalcid, *Mormoniella vitripennis*, Walker.

It was discovered in Australia in 1913 by Froggatt who saw in it a means of combating the sheep blow-flies there and had a special insectary built for breeding it. It was distributed as mentioned above and a careful study was made of its habits and life history.

Froggatt subsequently realized that it was not effective in controlling the flies. This was because the fly puparia are attacked and not the maggots, and only those puparia which lie exposed on the surface of the ground. Because the habit of the maggot is to burrow down into the soil before pupation over ninety per cent. of the puparia escape the attack of this parasite.

Several other Australian authors confirm this by saying that while the chalcid has been found nearly all over Australia the fly pest has been steadily increasing.

The parasite was discovered in South Africa by the present author in 1924 attacking the puparia of *L. sericata* under a carcase and later it caused a lot of trouble by upsetting breeding work and resistance experiments in our insectary.

In the literature it is commonly referred to as *Nasonia brevicornis* but Gahan (56) has recently shown that this is a synonym and that its correct name is *Mormoniella vitripennis*, Walker. It is very widely distributed throughout the world. In America it is

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* Note.—On 14th January, 1930, an interesting chalcid was found on a piece of meat in the insectary. The meat was infested with the maggots of *S. haemorrhoidalis*, Meigen, and the chalcid was attacking these by stabbing them with her ovipositor.

A careful examination of the parasite and comparison with the description and plate by Froggatt revealed the fact that it was undoubtedly *Chalcis calliphorae*. The parasite lived three days and many *S. haemorrhoidalis* and *C. chlorpyga* maggots were exposed to it. Many of these maggots appeared to be killed by it when it pierced them with its ovipositor but no further parasites developed in them. This is the only specimen of the parasite that has been seen by the author.

found throughout the United States and also in Hawaii. It is found practically all over Australia and New Zealand and in Europe and England and apparently it is well distributed over South Africa for it has been found wherever looked for.

At Grootfontein it becomes very abundant at certain times of the year, depending on the number of fly puparia available, and almost every puparium left exposed is attacked. The chalcid cannot dig into the soil and puparia covered with half an inch of loose sand are safe from its attack. This fact is used in our insectary to protect puparia from it.

Hosts.

M. vitripennis has been recorded from the following hosts:—

In Australia—

Pycrosoma rufifacies, Macqart.
Pycnosoma (calliphora) varipes, Macqart.
Anastellorhina (calliphora) augur, Linn.
Pollenia stygia, Fabr. (Calliphora villosa).
Calliphora erythrocephala, Meigen.
Lucilia sericata, Meigen.
Sarcophaga aurifrons, Macqart.
Ophyra nigra.

In America—

Musca domestica, Linn.

In England—

Musca domestica, Linn.
Lucilia sericata, Meigen.
Lucilia caesar, Linn.
Phormia azurea.
Calliphora erythrocephala, Meigen.
Calliphora vomitoria, Linn.

In France—

Calliphora erythrocephala, Meigen.
Phormia sordida.

Experimentally—

Gastrophilus equi, Fabricius.
Glossina morsitans, Westwood.

In South Africa the author has reared it from—

Lucilia sericata, Meigen.
Chrysomyia albiceps, Wiedemann.
Chrysomyia chloropyga, Wiedemann.
Chrysomyia marginalis, Wiedemann.
Calliphora croceipalpis, Jaenn.
Sarcophaga haemorrhoidalis, Meigen.
Wohlfahrtia spp.

Life-history.

The adult chalcids first make their appearance in the Karroo area about the middle of September. They are very active and have a jerky whirling flight. On alighting on the fly puparia they walk quickly over them exploring their surface by tapping with the tips of the antennae. They will not pay any attention to maggots or freshly formed puparia while still yellowish. The puparia must be properly hardened before the parasite will oviposit in them. In common with many chalcids, *M. vitripennis* has the habit of feeding at the punctures it makes with its ovipositor and according to Roubaud (139) it is necessary for it to do so before laying its first egg. When at rest the ovipositor folds up against the underside of the abdomen. In use its tip is caught against the surface of the puparium and pulled away from the chalcid's body, so that it sticks out from the base of the abdomen. In this way the parasite can press her ovipositor into the puparium most efficiently.

In the case of hairy maggot puparia such as *C. albiceps* considerable difficulty is often found in piercing the shell and several attempts have to be made. When finally pushed right in the parasite remains still for a minute or more. The ovipositor is then withdrawn for about half its length and again pushed in. This may be done several times. After the ovipositor is finally withdrawn a small drop of liquid exudes and the female immediately turns round and sucks it up. The whole process of oviposition lasts from 5 to 20 minutes.

The larvae of the parasite feed as external parasites on the fly pupae within the puparium and may be found as white, slightly curved grubs attached to the developing pupae along the sides of the abdomen and under the wings. The fly pupae usually develop until they are a grey colour and apparently almost ready to emerge as flies but then shrink up as the parasitic larvae devour them.

If there are many larvae in the puparium the fly is consumed until there is nothing left of it but a very delicate skin. Where few are present the pupa is found only slightly reduced in size and dried up.

The number of parasites that can develop in a single puparium varies with the species of fly. From 100 *L. sericata* puparia 1,401 adult parasites emerged, and 25 more were found dead in the puparia. The total number of exit holes in this case was 160. In the case of *C. albiceps* and *S. haemorrhoidalis* puparia the parasites are often trapped, being unable to escape through the tough wall of the puparium.

From one puparium of *S. haemorrhoidalis*, 52 adults emerged. Three emergence holes were made in this case.

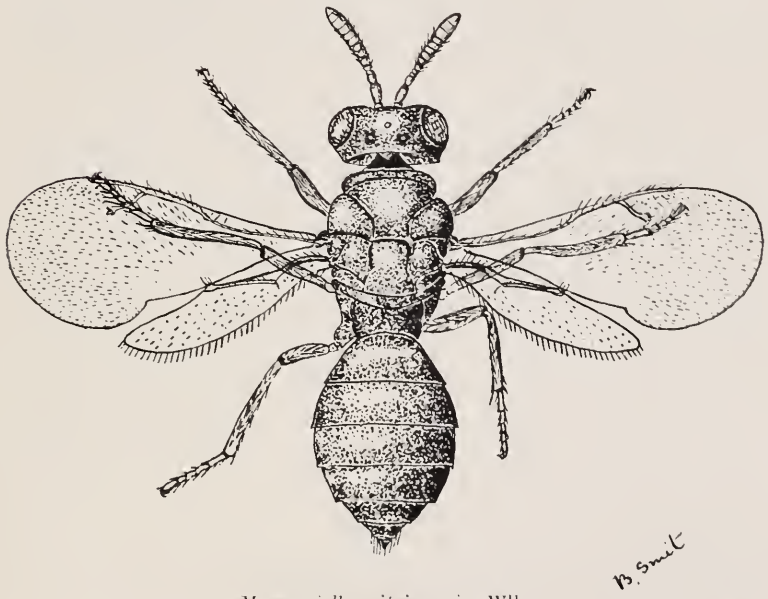
If puparia are over parasitized all the parasites may perish. In one case 113 parasite pupae were found dead in a single puparium of *S. haemorrhoidalis*. There was nothing left of the fly pupa but no adult parasites had emerged.

In puparia from which the parasites have emerged the cast pupal skins and pellets of excreta thrown out by the developing pupae can be easily found. The period of development of the parasite depends on temperature. In summer it is twelve days in *L. sericata* puparia being five days longer than the minimum pupal period of the fly.

Although this parasite may not be very efficient in destroying the sheep blow-flies in the field it has proved very beneficial in various types of maggot traps by destroying puparia lodged amongst old carcasses. Our most successful maggot trap is designed so that the full grown fly larvae when they crawl out of the carcasses, fall through wire netting into a poison solution. In practice it is found that some do not leave the carcasses but pupate amongst them. This is particularly true of *C. albiceps*. These puparia are, however, invariably destroyed by *M. vitripennis*.

It is interesting to note that Rouboud (139) in France succeeded in getting *M. vitripennis* to oviposit in puparia of the Horse bot fly, *Gastrophilus equi*, and the Tsetse fly, *Glossina morsitans*, and suggests its use against these most injurious flies. He discusses the question as to whether the parasite would live in the country infested with *Glossina*, but did not apparently know that it was common in South Africa.

PLATE VIII.



Mormoniella vitripennis, Wlk.

Alysia Manducator, Panzer.

The Braconid parasite *Alysia manducator*, Panz., is more likely to prove a factor in the control of the sheep blow-flies because it attacks the maggots of the flies rather than their puparia. It is for this reason that it has been recently introduced into Australia and is being widely distributed there.

There has been some difference of opinion as to the advisability of this action, however, and before the parasite is released in South

Africa a great deal more information about it than we have at present must be accumulated. Froggatt (47) in Australia protested against its introduction into that country on the ground that it is an indiscriminate parasite and is very partial to the beneficial Syrphidae. This does not seem to be proved and Myers (113), says that there are no grounds for such fears because the parasite confines itself to carcass-feeding maggots, it being only induced to oviposit by the presence of decomposing animal matter. This appears to be the case. In South Africa there are, however, other considerations of equal importance. We have in this country a Sarcophagid, *Wohlfahrtia cuvittata*, Villeneuve, whose maggots are predacious on the eggs of the brown swarm locust, and are parasitic on the locust nymphs. This locust is the most serious insect pest in South Africa and enormous sums of money have been spent in attempts to control it. It is believed that this "Locust-fly" as it is called, plays an important part in keeping the locust in check. The fly normally breeds in carrion on which it subsists between locust outbreaks, and it is feared that *A. manducator* might attack its maggots. If this is so it is very doubtful whether the liberation of Alysia in South Africa would be wise, no matter what effect it might have on the blow-flies. The parasite was therefore studied in strict confinement.

Introduction into South Africa.

Eight hundred parasitized *L. sericata* puparia were sent from England on the 22nd November. They were kept in cold storage on the boat and at Capetown until the 12th February after which they were sent on to Grootfontein. The delay at Capetown was due to the author's absence from Grootfontein at the time.

On opening the package one parasite was found to have emerged and was still alive but died the next day. The puparia appeared healthy although having a musty smell from the damp sand in which they had been packed. No further parasites emerged until February 19th when one more appeared and on February 28th twenty adult *A. manducator* emerged. They appeared normal and active.

The parasites continued to emerge until March the 8th when a total of 206 had come out. Some of these died almost immediately after emerging but most of them lived until the middle of the month and the last one died on March 25th. They were fed regularly each day with sugar water. Full grown *L. sericata* maggots were exposed to the parasites as soon as they appeared. They seemed to pay no attention to maggots crawling on clean sand in a dish but explored a putrid piece of meat in which maggots were feeding. They did not, however, succeed in parasitising the maggots until the third day after they had emerged.

On the second day copulation was observed and the parasites were watched attacking the maggots. Their persistance in this is remarkable and has been noted by other authors. The female crawls amongst the wriggling maggots in the rotting meat until her wings are covered with slime and put completely out of action. She holds on to the maggot she is trying to parasitise while it struggles violently, often at the risk of her life but finally when the

ovipositor is inserted the maggot lies still being paralysed by a poison injected into it. Myers has described this and the way in which the heartbeat of the maggot is arrested.

The period of development of the parasite appears to vary enormously. From maggots in which eggs had been laid on March the 15th, some adult *A. manducator* emerged on April the 23rd. These were the first adults of the second generation obtained, while in some puparia, the larvae of which were parasitised on March the 3rd, full grown larvae of Alysia in their cocoons were found on July 8th, more than four months later.

The percentage of maggots parasitised has been disappointing in spite of all our efforts to provide suitable conditions. Altogether 2,389 *L. sericata* maggots were exposed to the parasites. They were put into the jars containing the parasites with the meat in which they had developed when full grown but still feeding in the meat and they were left in the jars two and, sometimes, three days.

From these maggots 1,402 flies emerged. 530 of the maggots shrivelled up and died before pupating and 352 deformed puparia appear to have died at various stages of their development. Only 40 *A. manducator* adults emerged from all the puparia that developed from the maggots exposed to the first generation of the parasites.

From some of the maggots which were stung by the parasites normal flies emerged but there is reason to believe that the high mortality amongst the maggots before they pupated and amongst the puparia is partly due to the attack of the parasites.

Other species of fly maggots were exposed to the parasites and although these tests were only preliminary some interesting results were obtained.

A. manducator attacks the maggots of *C. albiceps* (not the *C. rufifacies* of Australia) and we have reared the adults from the puparia of this sheep blow-fly. 619 *C. albiceps* maggots were exposed to the parasites but only 19 adults emerged from these.

The parasites seemed to have great difficulty in piercing the skin of the hairy maggots partly on account of its thickness and partly because of the activity of the maggots. Several of the parasites were killed outright by the maggots while trying to oviposit in them. They were caught and held by the maggots as they curled up and pierced by their sharp mouth hooks.

The shortest period of development of *A. manducator* in *C. albiceps* puparia was 38 days.

It is not yet known whether the parasite will complete its development in the puparia of the sheep blow-fly, *C. chloropyga*. Its maggots were exposed to *A. manducator* and were attacked, eggs apparently being laid in them but no adult parasites emerged.

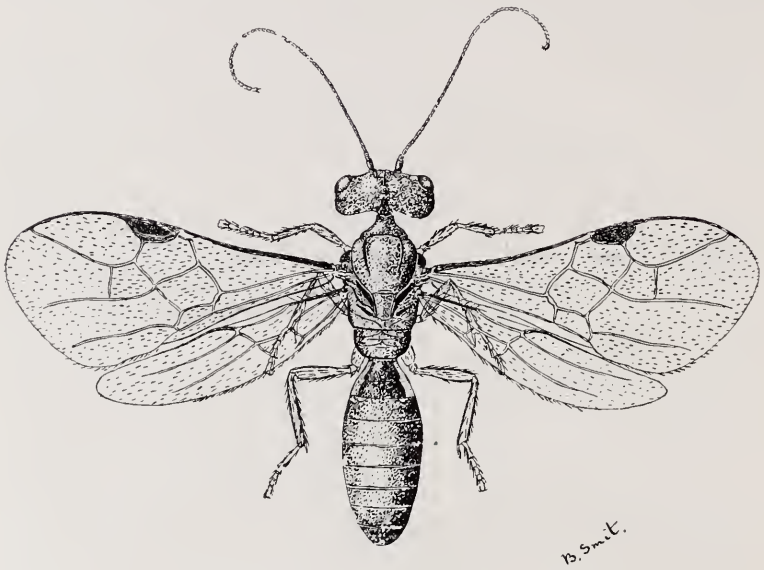
Six maggots of *S. haemorrhoidalis* were attacked but all shrivelled up except one which succeeded in pupating, but died soon after. From a check lot which were not exposed to the parasite all the flies emerged.

Six *Wohlfahrtia curittata* maggots were exposed to the parasites for three days but without result. Four perfect flies emerged and two fly pupae died in their puparia.

The parasite has now died out and it will be necessary to import a fresh consignment in order to continue this work. No adults emerged in the spring although a number of the parasite larvae spun cocoons in fly puparia in the autumn and pupated. It is believed that our climate is too dry for the successful development of this species. Morgan in a recent article states that similar difficulties have been experienced in Australia and that the dry climate is detrimental to it there. Our average rainfall is only 13 inches a year and the atmosphere becomes extremely dry during the winter and spring.

From our experience with these parasites it appears that very little reliance can be placed upon them as checks to the ravages of the sheep blow-flies.

PLATE IX.



Alysia manducator, Panz.

CONCLUSION.

Because of the enormous powers of reproduction which the blow-flies possess, the most important control measure is the destruction of their breeding places and this entails the proper disposal of all carcasses. Burying of carcasses is not effective and they are best used as bait in large fly traps. This method has met with remarkable success throughout South Africa.

These studies have led, among other things, to the conclusion that crutching is the best way of protecting the sheep and that for dressing infested sheep oil mixtures containing volatile substances such as petrol, benzine or carbon bisulphide to kill the maggots and repellents, such as pyridine or pine tar oil are most effective.

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There is a vast amount of literature on the subject of the blow-fly, and it is not proposed to attempt to give a complete bibliography in the present paper. A complete card bibliography of articles on the subject, published since about 1900, has been made by the author and this consists of 273 references. Only those dealing most directly with the phases of the problem which are discussed in this paper are given, and they are lettered according to the phase chiefly dealt with.

The references are arranged according to name of author in alphabetical order.

The lettering corresponding to the arrangement of chapters in the present work is as follows:—

The general problem of sheep blow-fly	G.
Classification	C.
Records of myiasis	M.
Distribution	D.
Seasonal distribution	S.D.
Habits	H.
Baits and repellents	B.R.
Life histories	L.
Morphology of maggots	M.M.
Destruction of maggots	D.M.
Parasites	P.

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The Toxicity of the Pupae of the Moth *Nudaurelia cytherea.*

By D. G. STEYN, B.Sc., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

A QUANTITY of the above live pupae of the moth *Nudaurelia cytherea* (see photograph) was forwarded by Mr. McIntyre, Government Veterinary Officer, George, with the remark that they are suspected of having caused death in pigs. The caterpillar of this moth has been causing enormous damage to the pine plantations in the neighbourhood of George. Owners have been advised to run pigs in the plantations in order to destroy the caterpillars and pupae, which are eaten freely by pigs with the result that mortality occurred in these animals. The symptoms described were dullness, inappetence and death within three days.

The pupae weighed from 8.0 to 14.0 grams, the average weight being approximately 11.0 grams.

The following experiments were conducted at Onderstepoort with the fresh live pupae.

RABBITS.

One rabbit received 100 grams of minced pupae per stomach tube at 11 a.m. on 28.1.31, and another 100 grams at 4 p.m.

Result: Rabbit died the following night.

Post-mortem Appearances: Partly decomposed; white froth exuding from both nostrils; hyperaemia and oedema of the lungs; hyperaemia of the liver and kidneys; pronounced hyperaemia of the gastric mucosa.

One rabbit received 25 grams of minced pupae per stomach tube at 11.30 a.m. on 29.1.31.

Result: Rabbit died the following night after having exhibited listlessness and an accelerated respiration and heart beat.

Post-mortem Appearances: White froth exuding from both nostrils; both ventricles of the heart markedly dilated with coagulated blood; pronounced hyperaemia and oedema of the lungs and pronounced hyperaemia of the gastric mucosa.

One rabbit received 50 grams of minced pupae per stomach tube at 11.35 a.m. on 29.1.31.

Result: Listlessness, accelerated heart beat and respiration; dead within ten hours after dosage.

Post-mortem Appearances: White froth exuding from both nostrils; pronounced dilation of both ventricles of the heart; a pronounced hyperaemia and oedema of the lungs, and intense hyperaemia of the gastric mucosa.

One rabbit received 100 grams of minced pupae per stomach tube at 11.40 a.m. on 29.1.31.

Result: Listlessness; accelerated heart beat and respiration and death within eight hours after dosage.

Post-mortem Appearances: Partly decomposed; white froth exuding from both nostrils; pronounced dilation of both ventricles of the heart, and a marked hyperaemia and oedema of the lungs.

Pupae of *Nudaurelia cytherea* (natural size).

One rabbit received 5.0 grams of minced pupae per stomach tube at 11 a.m. on 30.1.31.

Result: 31.1.31, listless; not feeding; laboured respiration and accelerated heart beat. Given another five grams of pupae; 1.2.31, very apathetic; not feeding; laboured respiration and accelerated heart beat; 2.2.31, above symptoms more pronounced; 3.2.31, died over night.

Post-mortem Appearances: White froth exuding from both nostrils and mouth; dilation of both ventricles of the heart; pronounced hyperaemia and oedema of the lungs, and hyperaemia of the gastric mucosa.

One rabbit received 10 grams of minced pupae per stomach tube at 11.5 a.m. on 30.1.31.

Result: 31.1.31, apathetic; not feeding; accelerated respiration and heart beat; 1.2.31, died over night.

Post-mortem Appearances: As above.

One rabbit received 15 grams of minced pupae per stomach tube at 11.10 a.m. on 30.1.31.

Result: 31.1.31, listless; not feeding; laboured respiration and accelerated heart beat. Received another 15 grams; 1.2.31, died previous night.

Post-mortem Appearances: White froth exuding from both nostrils and mouth; dilatation of both ventricles of the heart; marked oedema and hyperaemia of the lungs and hyperaemia of the gastric mucosa.

Pigs.

Pig 722 (about four months old): Starved for 24 hours and offered 100 grams (= 8 pupae) of pupae on 2.2.31.

3.2.31: Nothing eaten.

4.2.31: Fed milk and crushed mealies and two hours later received 100 grams of minced pupae per stomach tube at 9.30 a.m.; within half an hour laboured respiration, apathetic, pronounced salivation and retching.

5.2.31: Vomited profusely previous night, and appeared normal; received another 100 grams of pupae with the result that profuse salivation and vomition occurred within three hours after dosage.

6.2.31: Feeding and apparently in normal health. Received another 100 grams of fresh pupae which within half an hour caused salivation and emesis.

7.2.31: Very apathetic and laboured respiration.

8.2.31: Very apathetic and laboured respiration.

9.2.31: Condition unchanged; received another 200 grams (=16 pupae) of pupae with the result that pronounced salivation, retching and emesis occurred within one and a half hours after dosage; the animal appeared extremely exhausted.

10.2.31: Very apathetic; pronounced laboured respiration.

11.2.31: Condition improved.

12.2.31: Apparently healthy. The drenching had to be discontinued as no more live pupae were left, the rest being decomposed.

Pig 723 (four months old): Starved for twenty-four hours and offered 200 grams of the pupae on 2.2.31.

3.2.31: Nothing eaten.

4.2.31: Nothing eaten. Fed milk and crushed mealies and two hours later received per stomach tube 200 grams (= 16 pupae) of minced pupae at 9.30 a.m.; vomited profusely within half an hour; completely exhausted; pronounced laboured respiration and inappetence.

5.2.31: Died previous night.

Post-mortem Appearances General cyanosis; subcutaneous blood vessels distended; heart in systole; subendocardial haemorrhages in left ventricle; hyperaemia of the lungs and liver and a very severe acute catarrhal gastro-enteritis with haemorrhages in the gastric mucosa.

Both pigs were very wild, so that there was no object in taking the pulse count.

Solubility Characters of the Toxin.

A. *Absolute Alcohol Extract.*

150 Grams of minced pupae extracted with an equal weight of absolute alcohol for five hours. The filtrate was evaporated down to 1 c.c. (= 10 grams of pupae) and injected subcutaneously into guinea-pigs.

One guinea-pig: 0.5 c.c. (= 5 grams pupae).

Result: Remained healthy.

One guinea-pig: 3.0 c.c. (= 30 grams pupae).

Result: Remained healthy.

One guinea-pig: 10 c.c. (= 100 grams pupae).

Result: Remained healthy.

B. *Ethereal Extract.*

150 Grams of minced pupae extracted with an equal weight of ether for five hours. The filtrate was evaporated down to 1 c.c. (= 10 grams of pupae) and injected subcutaneously into guinea-pigs.

One guinea-pig: 0.5 c.c. (= 5 grams pupae).

Result: Within five hours after injection the animal exhibited restlessness, pronounced laboured respiration and an extremely accelerated heart beat. Died within sixteen hours after injection.

Post-mortem Appearances: General cyanosis; both ventricles of the heart markedly dilated and distended with coagulated blood, and hyperaemia of the lungs. There were no signs of irritation at the point of injection.

One guinea-pig: 3.0 c.c. (=30 grams pupae).

Result: The above symptoms appeared within two hours after injection and death followed ten hours later.

Post-mortem Appearances: As described above. There were no signs of irritation at the point of injection.

One guinea-pig: 10.0 c.c. (= 100 grams pupae).

Result: Symptoms as described above appeared within one and a half hours after dosage and death occurred eight hours after dosage.

Post-mortem Appearances: As described above. There were no signs of irritation at the point of injection.

C. *Chloroform Extract.*

150 Grams of minced pupae were extracted with an equal amount of chloroform for five hours. The filtrate was evaporated down to 1 c.c. (= 10 grams of pupae) and injected subcutaneously into guinea-pigs.

One guinea-pig: 0.5 c.c. (= 5 grams of pupae).

Result: Within eight hours symptoms as described above appeared, death occurring within fifteen hours after injection.

Post-mortem Appearances: As described above. No signs of irritation at the point of injection.

One guinea-pig: 3.0 c.c. (= 30 grams of pupae).

Result: Symptoms as described above appeared within five hours and death occurred within twelve hours after injection.

Post-mortem Appearances: As described above. No signs of irritation at the point of injection.

One guinea-pig: 10 c.c. (= 100 grams of pupae).

Result: Symptoms as described above appeared within three hours and death took place ten hours after injection.

Post-mortem Appearances: As described above. No signs of irritation at the point of injection.

D. *Distilled Water Extract.*

150 Grams of minced pupae extracted with an equal weight of distilled water for five hours. The filtrate was evaporated down to 1 c.c. (= 10 grams of pupae) and injected subcutaneously into guinea-pigs.

One guinea-pig: 0.5 c.c. (= 5 grams pupae).

Result: The above symptoms appeared within three hours and death occurred within sixteen hours after injection.

Post-mortem Appearances: As described above. No signs of irritation at the point of injection.

One guinea-pig: 3.0 c.c. (= 30 grams pupae).

Result: The above symptoms appeared within half an hour and death occurred within one and a half hours after injection.

Post-mortem Appearances: As above. Slight gelatinous infiltration of the subcutaneous tissues at the point of injection.

One guinea-pig: 5.0 c.c. (= 50 grams pupae).

Result: The above symptoms were exhibited within half an hour and death occurred within two hours after injection.

Post-mortem Appearances: As described above. Slight gelatinous infiltration of the subcutaneous tissues at the point of injection.

TABLE.

EFFECTS OF THE PUPAE OF *Nudaurelia cytherea* ON PIGS, RABBITS AND GUINEA-PIGS.

Species of Animal.	Quantity of Fresh Pupae Given.	Form in which Pupae were Administered.	Method of Administration.	Result.
Rabbit.....	5 grams on 30/1/31.....	Minced.....	Per stomach tube..	Died within 60 hours after second dose.
	5 ,, 31/1/31.....	,, ...	,, ,, ..	
Rabbit.....	10 grams.....	,, ...	,, ...	Died within 36 hours after dosage.
Rabbit.....	15 grams on 30/1/31.....	,, ...	,, ,, ..	Died within 12 hours after second dose.
	15 ,, 31/1/31.....	,, ...	,, ,,	
Rabbit.....	25 grams.....	,, ...	,, ,, ..	Died within 12 hours after dosage.
Rabbit.....	50 grams.....	,, ...	,, ,, ..	Died within 12 hours after dosage.
Rabbit.....	100 grams.....	,, ...	,, ,, ..	Died within 8 hours after dosage.
Rabbit.....	100 grams at 11 a.m. on 28/1/31	,, ...	,, ,, ..	Died within 5 hours after dosage.
	100 grams at 4 p.m. on 28/1/31	,, ...	,, ,,	
Pig 722.....	100 grams on 4/2/31.....	,, ...	,, ,, ..	After each dose animal vomited and recovered.
	100 ,, 5/2/31.....			
	100 ,, 6/2/31.....			
	200 ,, 9/2/31.....			
Pig 723.....	200 grams.....	,, ...	,, ,, ..	Vomited profusely and died within 18 hours after dosage.
Guinea-pig.....	5 grams equivalent.....	Absolute alcoholic extract	Subcutaneously.....	Remained healthy.
Guinea-pig.....	30 ,, ,, ...	,, ,,	,, ...	,, ,,
Guinea-pig.....	100 ,, ,, ...	,, ,,	,, ...	,, ,,
Guinea-pig.....	5 grams equivalent.....	Ethereal extract....	,, ...	Died within 16 hours after injection.
Guinea-pig.....	30 ,, ,, ...	,, ,, ...	,, ...	Died within 12 hours after injection.
Guinea-pig.....	100 ,, ,, ...	,, ,, ...	,, ...	Died within 8 hours after injection
Guinea-pig.....	5 ,, ,, ...	Chloroform extract.	,, ...	Died within 16 hours after injection.
Guinea-pig.....	30 ,, ,, ...	,, ,, .	,, ...	,, 12 ,, ,,
Guinea-pig.....	100 ,, ,, ...	,, ,, .	,, ...	,, 10 ,, ,,
Guinea-pig.....	5 ,, ,, ...	Distilled water extract	,, ...	Died within 16 hours after injection.
Guinea-pig.....	30 ,, ,, ...	,, ,,	,, ...	,, $11\frac{1}{4}$,, ,,
Guinea-pig.....	50 ,, ,, ...	,, ,,	,, ...	,, 2 ,, ,,

Discussion.

Seven rabbits were drenched with quantities of fresh pupae ranging from 10 grams to 200 grams with fatal results. 200 Grams of pupae caused death in a 4-months-old pig within eighteen hours after dosage in spite of the fact that the animal vomited profusely within a short time after dosage. The symptoms and post-mortem appearances were those of a severe irritant. In another 4-months-old pig quantities of 100 grams and 200 grams of pupae caused transient emesis, apathy, inappetence and laboured respiration. In both pigs vomition occurred in spite of the fact that they had been well fed about two hours prior to dosage.

The toxin is very soluble in ether, chloroform and distilled water, and insoluble in absolute alcohol. No definite statements can be made as to the relative solubility of the toxin in ether, chloroform and distilled water, as the former two solvents mixed with the minced pupae in such a manner that only a small quantity of filtrate was obtained. In the case of the distilled water and absolute alcohol the filtrate amounted to about 90 per cent. of the original volumes of solvent added to the minced pupae.

Conclusions.

1. The pupae of the moth *Nudaurelia cytherea* proved to be toxic to rabbits and pigs on oral administration, and to guinea-pigs on subcutaneous administration of the ethereal, chloroform and aqueous extracts.

2. The toxin is soluble in ether, chloroform and distilled water, but not in absolute alcohol.

3. The toxin is a severe gastro-intestinal irritant.